Original Article

Transcutaneous light penetration of simultaneous superpulsed and multiple wavelength photobiomodulation therapy in living dog tissue

Somphong Hoisang[1] Suvaluk Seesupa[2] Supranee Jitpean[2] Naruepon Kampa[2*]

Abstract

Photobiomodulation therapy (PBMT) is used as an adjunctive therapy for wound healing, pain relief and rehabilitation. The penetration of light to the target tissue is the crucial factor. The aim of this study was to investigate the potential penetration of simultaneous superpulsed and multiple wavelength (SPMW) PBMT on living tissue in dogs. Twenty client-owned dogs were anesthetized and underwent abdominal surgery. The PMBT device was set at 50 Hz and delivered light for 60 sec at different distances; at 0 cm (contact) and 1 cm tissue-device distance (non-contact). The power meter was placed and measured the mean output power (MOP) under the skin tissue only and the combination of skin and muscle tissue. The average thickness of skin was 2.97 mm (ranging from 1.7 to 4.3 mm) and the combination of skin and muscle tissue thickness was 15.87 mm (ranging from 10.0 to 30.0 mm). For skin tissue, MOP was found to be at 13.00±4.99 mW and 3.47±1.79 mW using the skin contact and non-contact mode, respectively. For the combination of skin and muscle tissue, MOP was found to be at 1.46±1.11 mW and 0.35±0.51 mW using the skin contact and non-contact mode, respectively. The MOP of skin contact was significantly greater than the non-contact both on skin tissue only and the combination of skin and muscle tissue (P<0.05). Based on these results, transcutaneous SPMW PBMT with a preset parameter of 50 Hz has been proven to be appropriate for target tissue thickness up to approximately 30.0 mm.

Keywords: combined wavelength laser, low intensity laser therapy, penetration, photobiomodulation therapy, transmission

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Introduction

Photobiomodulation therapy (PBMT) is the use of a non-ionizing form of light including lasers, light-emitting diodes and broadband light in the visible and near-infrared spectrum (Anders *et al*., 2019). The mechanism of photobiomodulation (PBM) can be explained by the light photon being absorbed into cytochrome c oxidase, which acts as an endogenous chromophore in the mitochondrial membrane and then increases cellular adenosine triphosphate production as a universal fuel inside living cells (Karu, 2010). These mechanisms drive all biological reactions and change the concentration of reactive oxygen species, intracellular calcium ions and nitric oxide which leads to the activation of transcription factors (de Freitas and Hamblin, 2016).

PBMT is used as an adjunctive therapy for tissue repair, pain reduction and rehabilitation (Millis and Saunders, 2014). In recent decades, most studies have investigated the efficiency effects of a single wavelength of PBMT (Lucroy *et al*., 1999; Draper *et al*., 2012; Bennaim *et al*., 2017). Clinical studies in dogs have shown beneficial effects of PBMT, such as in increasing bone repair (Menezes *et al*., 2016), reducing postoperative recovery time (Draper *et al*., 2012) and increasing apexogenesis (Bahman *et al*., 2020). However, several studies have reported that PBMT has no influence on acute wound healing (Kurach *et al*., 2015; Gammel *et al*., 2018) and also no effect on pain and hindlimb function following tibial plateau leveling osteotomy (Kennedy *et al*., 2018). PBM efficiency effects are influenced by various parameters, including wavelength, power density, energy density, mode of operation, irradiation time and therapeutic technique (da Fonseca, 2019). In consideration of various PBMT devices and no standardized parameters, the different outcomes may result from light wavelengths and the potential ability to penetrate into the target tissue (Tunér and Hode, 1998).

Currently, the combined wavelength PBMT is emerging as a new approach for an alternative therapy. Evidence suggests that multiple-wavelength radiation produces more therapeutic advantages than single wavelength radiation (Lima *et al*., 2020). SPMW PBMT is a portable therapeutic device which simultaneously delivers red light (660 nm), broadband light (875 nm) and superpulsed light (905 nm) and recently has become available for veterinary use. SPMW PBMT has positive results on decreasing nonspecific knee pain in humans (Leal-Junior *et al*., 2014), and decreasing pain intensity and postoperative arthroplasty inflammation in humans (Langella *et al*., 2018).

Light penetration is considered to be a crucial factor for PBMT in the target tissue (Pryor and Millis, 2015). Several studies have investigated the penetration potential of single wavelength superpulsed light in rat tissue. Irradiation of superpulsed 904 nm wavelength with a peak power of 20 W, MOP of 60 mW and a frequency of 6 kHz on cadaver Sprague-Dawley rat skin showed a penetration potential at 38%-58.0% of the initial setting (Joensen *et al*., 2012). With the same parameter settings, superpulsed 904 nm wavelength in living Sprague-Dawley rats had an average of 19.94% and 4.01% of MOP detection for the skin only and a combination of skin including gastrocnemius muscle, respectively (Anders and Wu, 2016). At the same parameter setting in the human Achilles tendon, the MOP was found to have a range of 0.25%-0.38% during 150 secs of irradiation (Bordvik *et al*., 2017). Recently, in equines, MOP detection was found at 0.013% through living equine skin with a range from 3.2 to 6.8 cm tissue thickness (Luna *et al*., 2020).

The penetration potential of light has been studied primarily in human and experimental animal tissue. Because of the inherent difference in skin thickness, skin composition, skin blood supply, skin color and hair composition, however, translating these results in order to use them directly in dogs should be considered (Millis and Saunders, 2014). From our knowledge, information on the penetration potential of MOP of SPMW PBMT in dogs is lacking. Hence, the aim of this study was to investigate the penetration potential of SPMW PBMT on living tissue in dogs. This information will be useful for the PBMT in small animal practices. The hypothesis of this study was that SPMW PBMT irradiation can truly penetrate living dog tissue in the abdominal region.

Materials and Methods

Ethical approval: The study was approved by the Institutional Animals Care and Use Committee of Khon Kaen University under protocol number ACUC-KKU 37/60.

Samples: Twenty client-owned dogs with a range of 10-20 kg bodyweight, considered to be healthy dogs based on general physical examination and normal blood profiles, were included in this study. These dogs were anesthetized and underwent abdominal surgery for conventional ovariohysterectomy (OVH).

Instruments and parameter setting: The PBMT device (MR4 ActiVet Pro veterinary laser, Multi Radiance Medical, Solon, OH 44139, USA) was preset at 50 Hz according to the manufacturer's instruction manual for the treatment of inflammation and the parameter details are described in Table 1. To measure the MOP, a portable wireless power meter (PM160T, Thorlab®, USA) with a 1 cm^2 sensor aperture area covering an optical power range from 100 μW to 2 W, a wavelength from 190 to 10,600 nm and a measurement uncertainty of 5% was used. The device was sterilized by ethylene oxide gas sterilization (3M™Steri-Vac™ Sterilizer/Aerator 5XL, 3M health care, USA).

Surgical procedures and light measurements: Each dog was premedicated with diazepam 0.2-0.3 mg/kg intravenously and morphine sulfate 0.3-0.5 mg/kg was injected intramuscularly as preemptive analgesia. Anesthesia was induced with thiopental sodium 10 mg/kg intravenously and was maintained with 1-2% isoflurane in pure oxygen. The dog was positioned in dorsal recumbency and the hair was clipped over the ventral abdomen. The thickness of the abdomen wall including skin and muscle at the area of surgical incision for OVH was measured using ultrasound equipment (Logic® S7, GE, USA) at a frequency of 10 MHz with a broad-spectrum linear probe (9L-D) in

each dog (Fig. 1). A cephalic IV catheter was placed and lactated Ringer's solution was given at 10 ml/kg/h. Cefazolin sodium 25 mg/kg IV was administered as an antibiotic prophylaxis 30 mins prior to operation. The ventral abdomen was prepared for surgery by aseptic technique and a 3-4 cm caudal midline incision was made through the skin, subcutaneous fat and linear alba.

The PBMT probe was held stationary perpendicular to the skin approximately 0.5-1.0 cm from the incision edge and the light was delivered for 60 sec with the two different techniques; contact mode (0 cm) and non-contact mode (1 cm tissue-device distance) (Fig. 2A-B). The light energy (MOP) was firstly measured without tissue as the control prior to the experimental procedure. To measure the MOP through the dog's tissue, the power meter was placed underneath the rectus abdominis muscle at the same position as the PBMT probe for measuring light energy that passed through the combination of skin and muscle. Blunt dissection was performed to separate the skin tissue. Thereafter, the power meter was placed underneath the skin and the light penetration through skin only measured. The MOP was recorded using Thorlab optical power monitor software at 10, 20, 30, 40, 50 and 60 sec.

Table 1 The detailed parameter and technical specifications of simultaneous superpulsed and multiple wavelength photobiomodulation therapy.

Parameters	Specifications
Class	1M (IEC 60825-1:2007 and IEC 60825-1:2014)
Radiation wavelength	
Super pulsed laser radiation	905 nm
Broadband infrared radiation	875 nm
Visible red-light radiation	660 nm
Average mean output power	
Super pulsed laser radiation	Peak power 50 W
Broadband infrared radiation	250 mW
Visible red-light radiation	100 mW
Frequency	50 Hz
Pulse duration	110 (±20) nanosec
Magnetic field	35 (±10) mT
Irradiation time	60 sec
Aperture of the device	4 (±0.4) cm^2
Probe type	Cluster probe

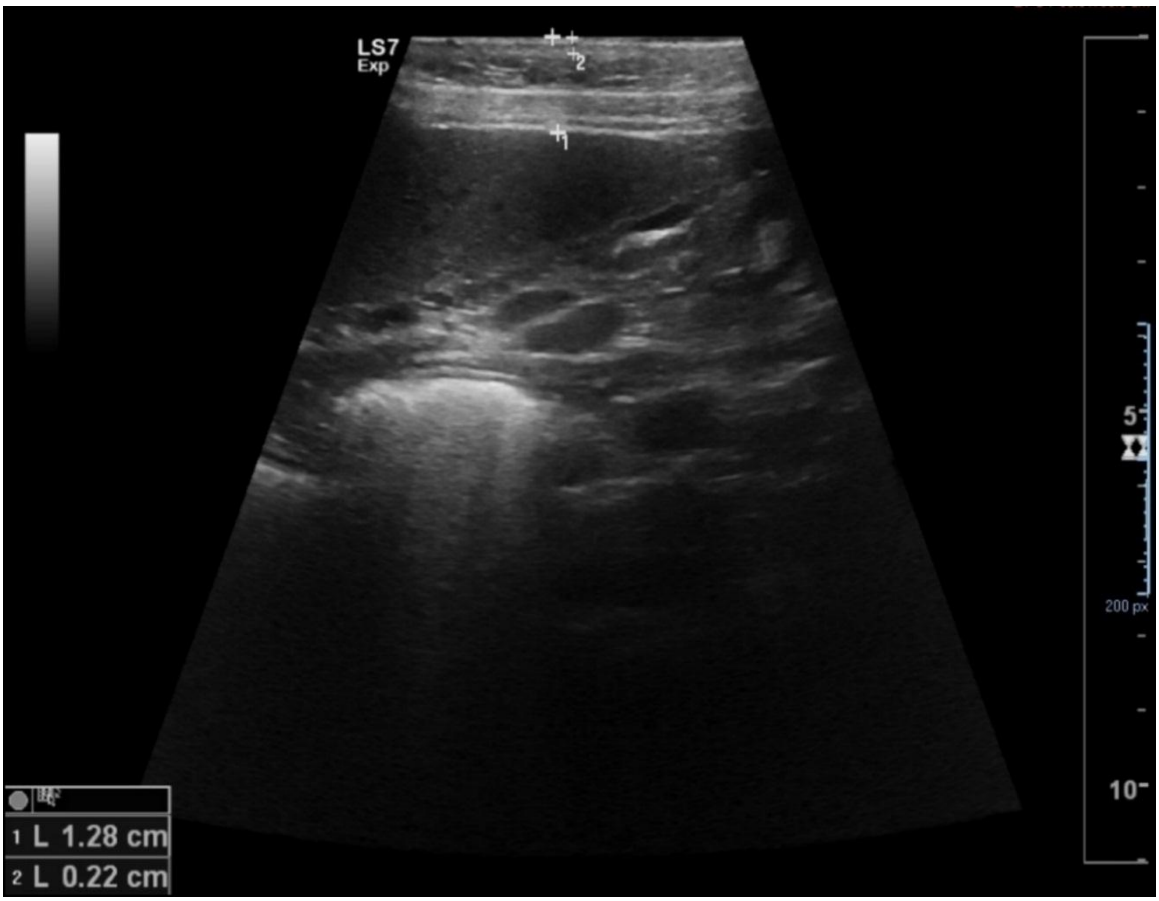

Figure 1 The skin thickness and combination of skin and muscle thickness were measured using ultrasonography.

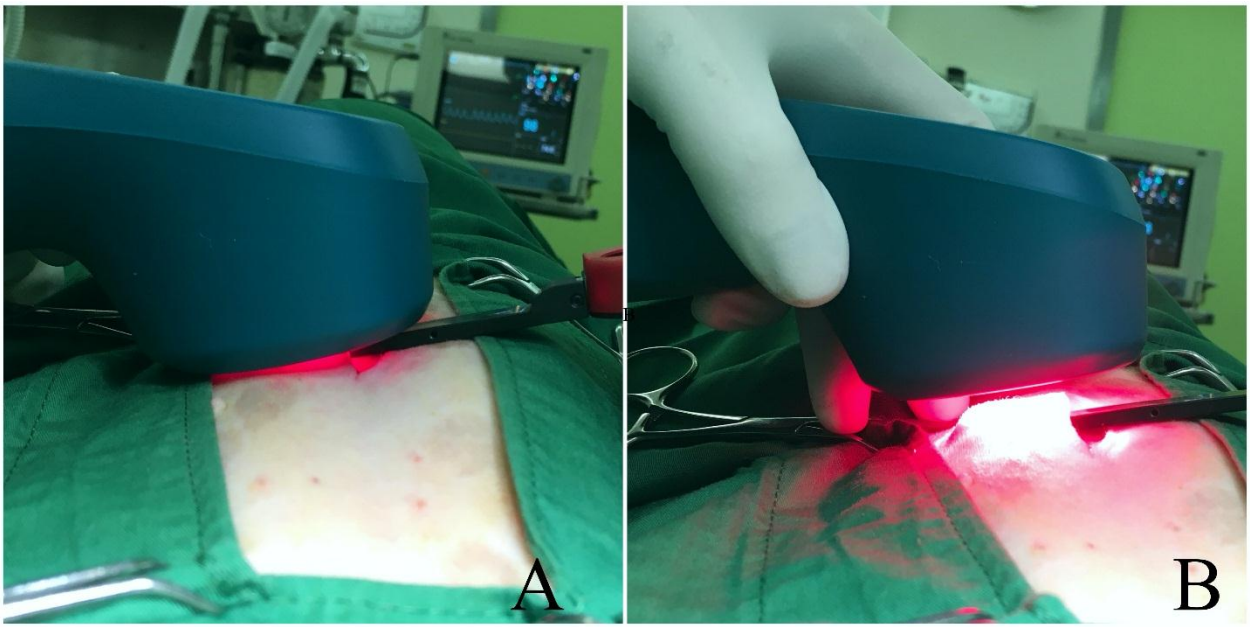

Figure 2 The light probe was held stationary at 90^0 to overlying skin. The power meter was inserted inside the tissue and measured MOP with skin contact (A) and 1 cm tissue-device distance (B).

Statistical analysis: The power penetration of light energy represented as the MOP was analyzed. The effect of tissue thickness (air, skin only and skin and muscle) and tissue-device distance on MOP was tested by two-way ANCOVA, covariate as tissue thickness. All significant differences of the tests were considered significant at $p<0.05$.

Results

Overall, there was no statistical difference in the tissue sample thickness between dogs. The average thickness of the skin was 2.97 mm (ranging from 1.7 to 4.3 mm) and for skin plus muscle was 15.87 mm (ranging from 10.0 to 30.0 mm). The MOP of SPMW PBMT using contact mode was significantly greater than non-contact mode, both in the control and the tissue tested (skin only and the combination of skin and muscle) ($P<0.05$). For the difference of tissue thickness, the MOP through the combination of skin and muscle significantly decreased compared to skin tissue only by contact at the skin surface ($P<0.05$) as shown in Table 2. Continuous measurements of MOPs were recorded for 60 secs; there was an increase of the MOP with time compared to the initial time point using skin contact irradiation. However, there were stable MOPs using the non-contact technique as shown in Fig. 3.

Table 2 An average of mean output power of light penetration of simultaneous superpulsed and multiple wavelength photobiomodulation therapy through skin tissue only, skin-muscle tissue in skin contact and skin non-contact (1 cm tissue-device distance).

Tissue samples (n=20)	**Tissue-probe distance**	**MOP±SD (mW)**
Skin tissue (2.97 mm)	Contact	13.00±4.99
	1 cm tissue-device distance	3.47±1.79
P-value		0.000*
Skin-muscle tissue (15.87 mm)	Contact	1.46±1.11
	1 cm tissue-device distance	0.35±0.51
P-value		0.0002*

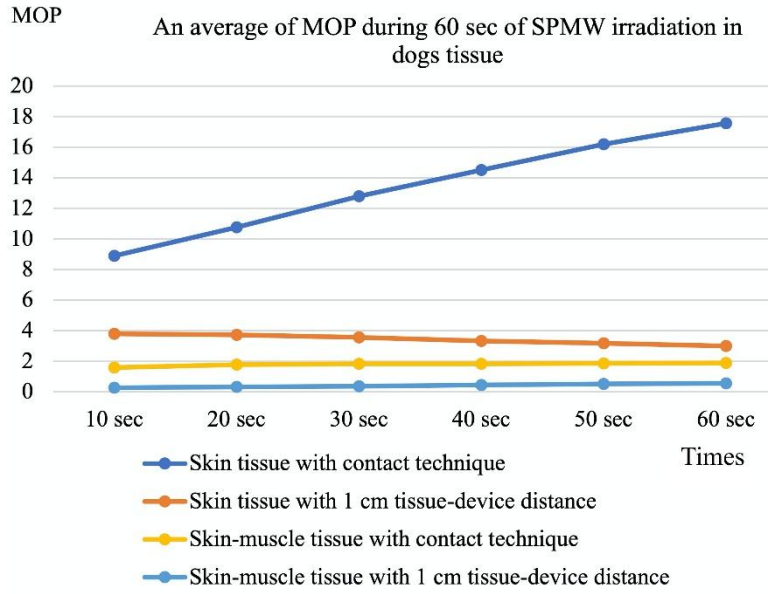

Figure 3 An average of mean output powers (MOP) penetrating dog tissue during 60 secs of simultaneous superpulsed and multiple wavelength photobiomodulation therapy.

Discussion

The results of this study show that the MOP using SPMW PBMT irradiation with a preset of 50 Hz can be detected through living dogs' abdominal tissue, where the thickness of the skin and muscle combination ranges from 10.0 to 30.0 mm. This finding indicates that the use of SPMW PBMT with the current setting would provide optimal therapeutic outcomes in the superficial condition and all other tissue conditions in which the target tissue does not exceed 30.0 mm in depth when using the contact mode. With the non-contact mode (1 cm tissue-device distance), the SPMW PBMT can be used up to a depth of 17.3 mm. The penetration potential in this study was less than the previous study in horses, which found that SPMW PBMT could penetrate through the living horse's skin in the cervical area with a tissue thickness ranging from 3.2 to 6.8 cm (Luna *et al*., 2020). The difference in results may be caused by the variation of subjects and composition of tissue, which likely affects the amount of light reflectance and light absorption. The amount of light that can be attenuated through the skin and muscle is in direct proportion to the tissue thickness (Enwemeka, 2001). In addition, a higher dense connective tissue and blood presenting in the muscle mainly affects light absorption (Stolik *et al*., 2000; Barbora *et al*., 2021). The difference in parameter setting plays an important role in influencing light penetration. It has been recently reported that irradiation of 808 nm light pulsed at 71.4 MHz has higher penetration than 808 nm light pulsed at 500 Hz (Barbora *et al*., 2021). Hence, increasing the pulse frequency would increase the depth of penetration in biological tissues.

As mentioned earlier, several studies have suggested that superpulsed 904-905 nm wavelength can penetrate deeper into biological tissue (Joensen *et al*., 2012; Bordvik *et al*., 2017; Luna *et al*., 2020). The most important factor which contributes to the light penetration into biological tissue is strongly influenced by the wavelength. In consideration of the tissue composition, melanin, whole blood, water and fat, are preferably absorbed at shorter wavelengths (Jacques, 2013). Therefore, a longer wavelength has relatively more penetration potential of the light energy through the tissue (Hashmi *et al*., 2010). Enwemeka (2001) indicated that infra-red 904 nm light had penetration potentially deeper than red 632.8 nm light in living rabbit tissue. In another study of human tissue, it was found that the near infrared light at 835 nm was the highest transmission light through human tissue compared to shorter wavelengths, which achieved depths of penetration of 3.72 mm, 3.46 mm, 1.63 mm, and 1.47 mm using 835 nm, 780 nm, 675 nm and 632.5 nm wavelength, respectively (Stolik *et al*., 2000). In addition, light irradiation with a higher power setting which has a higher number of light photons provided a sufficient therapeutic dosage in deeper tissue (Hamblin *et al*., 2018). Factors of wave emission, either continuous or pulse wave, can affect the light penetration. There has been a study that demonstrated that the penetration potential of the 830 nm continuous wave (CW) was significantly higher than pulsing 830 nm at 50 Hz in dog tissue (Kampa *et al*., 2020). Theoretically, light treatment with a high power setting and CW mode can generate more heat, causing tissue heating. However, the use of superpulsed PBMT that delivers the high peak power (1-70W) in a short time within one microsecond showed more penetration depth without thermal effects (Hashmi *et al*., 2010; Gupta *et al*., 2015). A previous study observed that the superpulsed 904 nm had the highest transmission through the cadaver tissue of rats and pigs. There was an average power of 42.32±0.82 mW, 32.40±0.70 mW, and 35.34±1.03 mW using superpulsed 904 nm, 660 nm CW, and 830 nm CW, respectively (Barbosa *et al*., 2020).

In veterinary medicine, regarding the difference in animal sizes and tissue components, there is a significant correlation between depth of penetration and tissue thickness, indicating light energy is being absorbed into tissue when tissue thickness increases (Hudson *et al*., 2013). It has been reported that

transcranial light transmission of 800 nm with a dose of 700 mW/cm^2 was 11.36% in rabbits, 21.24% in rats and 40.10% in mice with a skull thickness of 2.1 mm, 0.83 mm and 0.44 mm in rabbits, rats and mice, respectively (Lapchak *et al*., 2015). In consideration of the tissue composition, tissue pigment is directly related to light absorption. Di Giacomo and colleagues (2013) found that light of 904 nm penetrated to a tissue thickness of 1.8 cm, 3.6 cm and 5.5 cm in adult bovine muscle, pig muscle and chicken breast, respectively. This indicates that light was absorbed in the red meat regarding blood content and hemoglobin in the tissue. There was also a study that clearly showed a dark skin color had more absorption of light. It was suggested that the large amount of melanosome and melanin led to increasing light energy absorption (Mustafa and Jaafar, 2013). However, the present study did not observe the effect of skin color on the penetration potential.

Interestingly, the superpulsed 904 nm light showed a marked linear increase in the amount of light energy when the irradiation time was increased. This finding was similar to several previous studies, indicating that superpulsed 904 nm light had a higher percentage of MOP penetration linearly increasing over time in Sprague-Dawley rat skin tissue and in the human Achilles tendon (Joensen *et al*., 2012; Bordvik *et al*., 2017). These studies concluded that an increasing depth of penetration over time may result from photobleaching effects and tissue structural changes by strong pulsed (high peak power) irradiation. This is an important issue to consider; increasing radiation time might risk an overdosage of energy density for light treatment. Therefore, applying PBMT with sequential scanning or a point-to-point technique will provide an appropriate dose of energy density and avoid unwanted excessive light on the target area (Enwemeka, 2009; Pryor and Millis, 2015). It has also been suggested that increasing light penetration depth over time may be caused by an insufficient warm-up period (Anders and Wu, 2016). This study reported that transmission of superpulsed 904 nm light reached a stable output power after warming up the equipment for two-cycles of 5 mins each. The difference in the penetration potential of MOP may be caused by the difference in the tissue samples; cadaver tissue vs living tissue. Measurement of light transmission through cadaver tissue without blood circulation and with less tissue moisture could reduce the absorption coefficient and increase light penetration depth (Graaff *et al*., 1993).

Additionally, our study found that the MOP of SPMW PBMT was greater in skin contact mode than non-contact mode. This result was similar to a penetration study in cadaver dog tissues showing a skin contact application of 980 nm with 3.14 W/cm^2 had more penetration depth to the spinal canal in dogs compared to the non-skin mode (Piao *et al*., 2019). It has been stated that the MOP of the non-contact mode decreased correlating to the tissue-device distance, where light reflection influenced the light energy loss (Price *et al*., 2000). Hence, the use of PBMT with non-contact and increasing the tissue-device distance can cause less MOP compared to skin contact. However, light irradiation with non-contact can deliver to superficial target tissues such as skin and provide beneficial effects on treatment of open wounds. However, the incidence of reflected light or a scattered beam may contribute to the risk of eye injuries. In particular, light absorption in the retina causes permanent damage to central vision (Smalley, 2011). Transcutaneous light treatment with the skin contact mode can reduce unwanted light reflection and provide more safety. It can also provide better penetration depth to deep tissue injuries, such as tendinitis, bone healing, and osteoarthritis (OA). Hence, PBMT should be used with consideration based on the suitable parameters for therapeutic purposes and without forgetting the potential hazard to users, patients and other persons involved.

In another advantage of the SPMW PBMT, the equipment is designed as a cluster probe with an aperture of 4 cm^2 as shown in Fig. 4. A large radiation aperture provides a larger treatment area compared to the small probe used in single wavelength PBMT. In addition, the large probe size provides more light dispersion in tissues and results in a greater depth of penetration (Ash *et al*., 2017). Simultaneously delivered light including red light (660 nm), broadband light (875 nm) and superpulsed light (905 nm) provides more power and can also reduce treatment times. It has been stated that the combination of multiple wavelengths can achieve better therapeutic outcomes compared to the single wavelength PBMT and non-PBMT (Mendez *et al*., 2004). It is supposed that multiple wavelengths have a synergistic effect on the light absorption at the cellular level with different amounts of each wavelength and this may result in better outcomes (Lima *et al*., 2020). In another suggested technique to improve the light penetration, it is recommended to clip the hair and clean the skin with alcohol solution. However, cleaning with alcohol solution without hair clipping did not improve light penetration (Ryan and Smith, 2007). In our study, the experimental area was cleaned and hair was clipped, therefore the results should represent the true penetration potential. Partial or entire skin compression during the light irradiation showed an increase of light propagation depth. This effect may be explained as the compressed skin will reduce the scattering coefficient the same as the contact technique (Kwon *et al*., 2009).

As for the limitations of the study, the power meter did not measure the power of light directly but it is based on a thermal sensor as MOP. However, this technique is a reliable method and is widely used in clinical research. Another limitation of this study was that we did not measure the MOP through the target area/structure, such as the spine, hip, long bone, tendon, muscle which is difficult to perform in clinical practice. Further studies should be focused on measuring light energy with distinct wavelengths on other tissues. The information will provide benefit to produce therapeutic outcomes in specific conditions, including alleviating neck pain, back pain, bone healing, OA hip or tendinitis.

In conclusions, based on our results, the use of SPMW PBMT with a preset parameter of 50 Hz has sufficient light energy to produce PBM on the target tissue, in which the thickness can be up to approximately 30.0 mm. The irradiation technique

with skin contact mode of SPMW PBMT provides deeper tissue penetration. However, light treatment of superficial conditions, such as an open wound, can be applied using the non-contact mode with approximately 1 cm distance above the skin surface.

Conflict of interest statement: The authors declare no conflict of interest related to this report.

Figure 4 The cluster probe consisted of an aperture of 4 cm^2 and a concave tip (arrow).

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References

Anders JJ and Wu X. 2016. Comparison of light penetration of continuous wave 810 nm and superpulsed 904 nm wavelength light in anesthetized rats. Photomed Laser Surg. 34(9): 418-424.

Anders JJ, Arany PR, Baxter GD and Lanzafame RJ. 2019. Light-emitting diode therapy and low-level light therapy are photobiomodulation therapy. Photobiomodul Photomed Laser Surg. 37(2): 63-65.

Ash C, Dubec M, Donne K and Bashford T. 2017. Effect of wavelength and beam width on penetration in light-tissue interaction using computational methods. Lasers Med Sci. 32(8): 1909-1918.

Bahman S, Sara G, Somayeh H, Parvin T, Kalhori KAM, Mona S, and Reza F. 2020. Combined effects of calcium hydroxide and photobiomodulation therapy on apexogenesis of immature permanent teeth in dogs. J Photochem Photobiol B. 207: 111867.

Barbora A, Bohar O, Sivan AA, Magory E, Nause A and Minnes R. 2021. Higher pulse frequency of near-infrared laser irradiation increases penetration depth for novel biomedical applications. PLoS ONE. 16(1): e0245350.

Barbosa RI, de Jesus Guirro EC, Bachmann L, Brandino HE and de Jesus Guirro RR. 2020. Analysis of low-level laser transmission at wavelengths 660, 830 and 904 nm in biological tissue samples. J Photochem Photobiol B. 209: 111914.

Bennaim M, Porato M, Jarleton A, Hamon M, Carroll JD, Gommeren K and Balligand M. 2017. Preliminary evaluation of the effects of photobiomodulation therapy and physical rehabilitation on early postoperative recovery of dogs undergoing hemilaminectomy for treatment of thoracolumbar intervertebral disk disease. Am J Vet Res. 78(2): 195-206.

Bordvik DH, Haslerud S, Naterstad IF, Lopes-Martins RAB, Leal Junior ECP, Bjordal JM and Joensen J. 2017. Penetration time profiles for two class 3B lasers in In situ human Achilles at rest and stretched. Photomed Laser Surg. 35(10): 546-554.

de Freitas LF and Hamblin MR. 2016. Proposed mechanisms of photobiomodulation or low-level light therapy. IEEE J Sel Top Quantum Electron. 22(3): 7000417.

de Souza da Fonseca A. 2019. Is there a measure for low power laser dose? Lasers Med Sci. 34(1): 223-234.

Di Giacomo P, Orlando S, Dell'Ariccia M and Brandimarte B. 2013. Low level laser therapy: laser radiation absorption in biological tissues. Appl Phys A. 112(1): 71-75.

Draper WE, Schubert TA, Clemmons RM and Miles SA. 2012. Low-level laser therapy reduces time to ambulation in dogs after hemilaminectomy: a preliminary study. J Small Anim Pract. 53(8): 465-469.

Enwemeka CS. 2001. Attenuation and penetration of visible 632.8 nm and invisible infra-red 904 nm light in soft tissues. Laser Therapy. 13: 95-101.

Enwemeka CS. 2009. Intricacies of dose in laser phototherapy for tissue repair and pain relief. Photomed Laser Surg. 27(3): 387-393.

Gammel JE, Biskup JJ, Drum MG, Newkirk K and Lux CN. 2018. Effects of low-level laser therapy on the healing of surgically closed incisions and surgically created open wounds in dogs. Vet Surg. 47(4): 499-506.

Graaff R, Dassel ACM, Koelink MH, de Mul FFM, Aarnoudse JG and Zijistra WG. 1993. Optical properties of human dermis in vitro and in vivo. Appl Opt. 32(4): 435-447.

Hamblin M, Carroll J, Freitas L, Huang YY and Ferraresi C. 2018. Low-level light therapy: photobiomodulation. 1st ed. Bellingham, Washington, USA. SPIE Press. 388 pp.

Hashmi JT, Huang YY, Sharma SK, Kurup DB, De Taboada L, Carroll JD and Hamblin MR. 2010. Effect of pulsing in low-level light therapy. Lasers Surg Med. 42(6): 450-466.

Hudson DE, Hudson DO, Wininger JM and Richardson BD. 2013. Penetration of laser light at 808 and 980 nm in bovine tissue samples. Photomed Laser Surg. 31(4): 163-168.

Jacques SL. 2013. Optical properties of biological tissues: a review. Phys Med Biol. 58(11): R37-R61.

Joensen J, Ovsthus K, Reed RK, Hummelsund S, Iversen VV, Lopes-Martins RA and Bjordal JM. 2012. Skin penetration time-profiles for continuous 810 nm and superpulsed 904 nm lasers in a rat model. Photomed Laser Surg. 30(12): 688-694.

Kampa N, Jitpean S, Seesupa S and Hoisang S. 2020. Penetration depth study of 830 nm low-intensity laser therapy on living dog tissue. Vet World. 13(7): 1417-1422.

Karu T. 2010. Mitochondrial mechanisms of photobiomodulation in context of new data about multiple roles of ATP. Photomed Laser Surg. 28(2): 159-160.

Kennedy KC, Martinez SA, Martinez SE, Tucker RL and Davies NM. 2018. Effects of low-level laser therapy on bone healing and signs of pain in dogs following tibial plateau leveling osteotomy. Am J Vet Res. 79(8): 893-904.

Kurach LM, Stanley BJ, Gazzola KM, Fritz MC, Steficek BA, Hauptman JG and Seymour KJ. 2015. The effect of low-level laser therapy on the healing of open wounds in dogs. Vet Surg. 44(8): 988-996.

Kwon K, Son T, Lee KJ and Jung B. 2009. Enhancement of light propagation depth in skin: cross-validation of mathematical modeling methods. Lasers Med Sci. 24(4): 605-615.

Langella LG, Casalechi HL, Tomazoni SS, Johnson DS, Albertini R, Pallotta RC, Marcos RL, de Carvalho PTC and Leal-Junior ECP. 2018. Photobiomodulation therapy (PBMT) on acute pain and inflammation in patients who underwent total hip arthroplasty - a randomized, triple-blind, placebo-controlled clinical trial. Lasers Med Sci. 33(9): 1933-1940.

Lapchak PA, Boitano PD, Butte PV, Fisher DJ, Hölscher T, Ley EJ, Nuño M, Voie AH and Rajput PS. 2015. Transcranial near-infrared laser transmission (NILT) profiles (800 nm): systematic comparison in four common research species. PLoS ONE. 10(6): e0127580.

Leal-Junior ECP, Johnson DS, Saltmarche A and Demchak T. 2014. Adjunctive use of combination of super-pulsed laser and light-emitting diodes phototherapy on nonspecific knee pain: double-blinded randomized placebo-controlled trial. Lasers Med Sci. 29(6): 1839-1847.

Lima AMCT, da Silva Sergio LP and de Souza da Fonseca A. 2020. Photobiomodulation via multiple-wavelength radiations. Lasers Med Sci. 35(2): 307-316.

Lucroy MD, Edwards BF and Madewell BR. 1999. Low-intensity laser light-induced closure of a chronic wound in a dog. Vet Surg. 28(4): 292-295.

Luna SPL, Schoen A, Trindade PHE and da Rocha PB. 2020. Penetration profiles of a class IV therapeutic laser and a photobiomodulation therapy device in equine skin. J Equine Vet Sci. 85: 102846.

Mendez TMTV, Pinheiro ALB, Pacheco MTT, Nascimento PM and Ramalho LMP. 2004. Dose and wavelength of laser light have influence on the repair of cutaneous wounds. J Clin Laser Med Surg. 22(1): 19-25.

Menezes RF, Araújo NC, Carneiro VSM, Moreno LM, Guerra LAP, Santos Neto AP and Gerbi MEM. 2016. Assessment of the effects of laser photobiomodulation on peri-implant bone repair through energy dispersive x-ray fluorescence: A study of dogs. In *Mechanisms of Photobiomodulation*

Therapy XI. Hamblin, MT, Carroll, JD and Arany, P (Ed.). California, USA. Proceeding of SPIE Vol. 9695, 96950T [Online]. Available: https://www.spiedigitallibrary.org. Accessed March 21, 2021.

Millis DL and Saunders DG (2014). Laser therapy in canine rehabilitation. In: Canine rehabilitation and physical therapy. 2nd ed. Millis, DL and Levine, D (ed.). St. Louis: W.B. Saunders. 359-380.

Mustafa FH and Jaafar MS. 2013. Comparison of wavelength-dependent penetration depths of lasers in different types of skin in photodynamic therapy. Indian J Phys. 87(3): 203-209.

Piao D, Sypniewski LA, Dugat D, Bailey C, Burba DJ and DeTaboada L. 2019. Transcutaneous transmission of photobiomodulation light to the spinal canal of dog as measured from cadaver dogs using a multi-channel intra-spinal probe. Lasers Med Sci. 34(8): 1645-1654.

Price RB, Dérand T, Sedarous M, Andreou P and Loney RW. 2000. Effect of distance on the power density from two light guides. J Esthet Dent. 12(6): 320-327.

Pryor B and Millis DL. 2015. Therapeutic laser in veterinary medicine. Vet Clin North Am Small Anim Pract. 45(1): 45-56.

Ryan T and Smith RKW. 2007. An investigation into the depth of penetration of low level laser therapy through the equine tendon in vivo. Ir Vet J. 60(5): 295-299.

Smalley PJ. 2011. Laser safety: Risks, hazards, and control measures. Laser Ther. 20(2): 95-106.

Stolik S, Delgado JA, Perez A and Anasagasti L. 2000. Measurement of the penetration depths of red and near infrared light in human "ex vivo" tissues. J Photochem Photobiol B. 57(2-3): 90-93.

Tunér J and Hode L. 1998. It's all in the parameters: a critical analysis of some well-known negative studies on low-level laser therapy. J Clin Laser Med Surg. 16(5): 245-248.