Challenges of transcutaneous laser application for the potential of photobiomodulation of the spinal cord at the scale of a large companion animal

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ABSTRACT

Photobiomodulation (PBM) has been used successfully for the treatment of nervous system and has been demonstrated in the rodent model. In contrast, the percutaneous use of PBM to treat spinal cord of companion animals is expected to be challenging due to the significant attenuation of light energy as it travels through the thick and heterogeneous layers of tissue and bone to reach the level of the spinal cord. This pilot study was performed on a cadaverous dog to determine if the recommended bio-stimulatory treatment dose can be delivered to the spinal canal via percutaneous application of a clinically acceptable surface dose. The dose reaching the spinal canal after percutaneous application was measured at 980nm by using a miniature photo-diode sensor with a dose-response sensitivity of 1V per 1mW/cm² dose and a 2mm spherical isotropic fiber-optical diffusor probe. The two sensors were embedded in different longitudinal positions along the dorsal portion of the spinal canal just below the soft tissues and vertebral processes in a 40lbs cadaverous dog. The spinal cord was then accessed via a hemilaminectomy. Once embedded in the target tissue, 1W-10 W surface irradiation was applied. At the T12/13 and T13/L1 intervertebral disc positions, photo-diode sensors detected the intra-spinal dose above the noise floor at the 10W surface dose. A narrow treatment window for percutaneous PBM in large dog may exist only for the shallowest segment of the spinal cord, which may be important to avoid potential collateral photothermal effects. Works for simultaneous multi-site intra-spinal measurements are on-going.

Keywords: Low level light therapy; photobiomodulation; spinal cord; photon diffusion; transcranial, transcutaneous.

1. INTRODUCTION

Photobiomodulation (PBM), previously referred to as low-level light therapy (LLLT), has been investigated as a viable treatment for injuries and diseases of the central nervous system (CNS) in both animal models and clinical trials [1]. PBM has been shown to improve disease conditions of the CNS through the application of non-thermal and near-infrared (NIR) light which delivers photons to targeted tissues. The interaction of photons with one or more chromophores, including cytochrome C oxidase [2, 3], has been found to alter cellular function, resulting in increased cellular adenosine triphosphate (ATP) and reactive oxygen species (ROS) [4, 5]. This interaction ultimately results in beneficial cellular modulations including reduced swelling and edema.

Although the mechanisms of PBM are far from adequately resolved [6–8], there is a developing consensus that the bio-stimulatory effect of the non-thermal light is dose-dependent [9] and potentially multi-phasic [10]. For example, von Leden et al. [11] have reported that PBM with energy densities between 4 and 30 J/cm² induced expression of M1 markers in microglia, whereas markers of the M2 phenotype, including the mannose receptor CD206 and the metallopeptidase inhibitor 1, were observed at lower energy densities of 0.2–10 J/cm². Additionally, co-culture of PBM or control-treated microglia with primary neuronal cultures demonstrated a dose-dependent effect of PBM on microglial-induced neuronal growth and neurite extension [11]. The dose-dependence of PBM, on the other hand, strongly infers a minimal level of dose and irradiance to be reached at the treatment site for the targeted bio-stimulatory reactions to be enacted.
The dose-dependent nature of the effects of PBM must be considered in clinically relevant situations as the potential for collateral thermal damage of superficial tissues may result from high-energy density applications. The transcutaneously applied surface energy, to be delivered to deep tissues like CNS, is dictated by the light penetration gradient from the skin surface to the site of treatment. For transcranial application, as was tested on human cadaver heads, Tedford et al. [2] have shown that 808-nm wavelength light penetrated the scalp, skull, meninges, and brain to a depth of approximately 4 cm with an effective attenuation coefficient for the system of 2.22 cm⁻¹. An effective attenuation coefficient of 2.22 cm⁻¹ is equivalent to an approximate 10% transmission for every centimeter of tissue penetration. For a 4-cm depth of homogeneous tissue, this level of light penetration gradient will attenuate an entrance irradiance of 1 W/cm² to a terminal irradiance of 100 µW/cm², i.e., an overall transmission of only 0.01 %.

In addition to the extending demonstrations that transcranial PBM improves cerebral neurological functions, there are several studies proposing PBM as a potential therapy for promoting neuronal regeneration and functional recovery after spinal cord injury (SCI). For example, Byrnes et al. [12] applied 810-nm light of 150 mW transcutaneously to treat SCI on adult rats, with a surface irradiance of 0.53 W/cm². Byrnes’ study has reported a 6 % power penetration to the spinal cord depth, delivering a local irradiance of 31.8 mW/cm². This local irradiance, applied for a daily duration of 2,997 s and over 14 consecutive days, has shown to significantly increase axonal number and distance of regrowth, to return aspects of function to baseline levels, and to significantly suppress immune cell activation and cytokine/chemokine expression. Wu et al. [13] transcutaneously applied 810-nm light with the surface irradiance and total dosage the same as those in Byrnes’s study to acute SCI in rats caused by a contusion model and a dorsal hemisection model. For both models, there was a statistically significant axonal regeneration and functional recovery in the light-treated group compared to untreated control. Medalha et al. [14] evaluated the effects of PBM on bone healing using a tibial bone defect experimental model in SCI rats, using an 808-nm laser (power, 30 mW; irradiance, 1.7 W/cm²; energy density, 100 J/cm²; total energy, 2.8 J). The results of the histological and morphometric evaluation demonstrated that the light-treated SCI group showed a larger amount of newly formed bone compared to the untreated control group. Moreover, a significant immuno-expression of runt-related transcription factor 2 was observed in the light-treated SCI group. The results suggest that PBM accelerated the process of bone repair in rats with complete SCI.

The reports on PBM of the SCI of rats suggest the potential of PBM for improving similar conditions in human. Recently, Holanda et al. [15] demonstrated that minimally-invasive laser irradiation had resulted in an immediate decrease in low back pain after the laminectomy procedure similar to pain reduction caused by lidocaine injection. In Holanda’s study, a single treatment was administered percutaneously by a continuous wave (CW), 808-nm wavelength diode laser, with an output power of 100 mW delivered via a 600-µm optical fiber passing through an 18G needle placed in the second lumbar intervertebral foramen guided by fluoroscopy (beam spot size, 0.003 cm²; irradiance, 35 W/cm²; exposure time, 84 s; energy density, 2800 J/cm²; total energy, 8.4 J). When it comes to the potential of PBM of the spinal cord, a clinical protocol likely will have to achieve the total target dosage over multiple treatment areas, which favors non-invasive transcutaneous application of the treatment light. However, by referring to transcranial PBM, one could expect that transmitting bio-stimulatory dose to the spinal cord via application of safe surface dose will be challenging due to the depth of the spinal cord and the strong attenuation expected from the spinal structures. Additionally, a treatment protocol for PBM of the spinal cord likely should also address the potentially negative thermal effects on the peripheral regions of the target tissue when trying to treat deep conditions, based on personal communications.

No study has reported the skin-to-spine transmission of light at a human scale, a critical set of information that is needed for evaluating the clinical feasibility of PBM for improving spinal cord conditions. The specific objective of this pilot study was to provide the first quantitative insight regarding the amount of light that may be transmitted to the spinal canal of a large dog under a clinically acceptable surface power. The study being inherently limited due to the use of only one cadaverous dog, nonetheless offers an intra-subject comparison between transcutaneous transmission to the spinal canal and transcranial delivery, and an intra-subject evaluation of skin-to-spine transmissions at five vertebral regions. The intra-subject information could be useful to assessing the feasibility and a potential application window for delivering bio-stimulatory dose to the spinal cord via safe surface irradiation. As the information was relevant to a large dog, it has direct indication to PBM for companion animals and implication to PBM for humans as well.
2. MATERIALS AND METHODS

2.1 Photodiode sensor
A miniature photodiode sensor with calibrated response was provided by LiteCure LLC (Newark, DE, USA). The sensor has a silicon positive intrinsic negative (PIN) photodiode epoxied in a glass enclosure measuring 14 mm × 5 mm × 3 mm, as is photographed in Figure 1A. The photodiode was biased at 9 V by a battery, and the photodiode current was conditioned to produce 1 V per 1 mW/cm² irradiance at 900 nm, according to LiteCure. The light-sensitive panel of the photodiode had an effective receiving aperture of 120°, and the wire connecting the distal terminal of the photodiode was routed along the side opposite to the light-receiving panel before joining the wire connecting the proximal terminal of the photodiode. In all measurements, the photodiode sensor was positioned to have the sensitive panel facing against the line-of-sight from the source of irradiation to maximize the flux acceptance of the sensor. The photodiode sensor output was routed via coaxial cable and BNC connector to a PC oscilloscope (PicoScope 3203D; Pico Technology, St. Neots, United Kingdom) that samples at a 1 GS/s rate with 8-bits digital resolution when controlled by a laptop computer through USB 2.0 protocol. The vendor-provided PicoScope 6 user interface displayed the real-time voltage reading from the sensor-input as a continuous trace that was updated at a user-controlled time-resolution (over a range of 2 ns/div – 5000 s/div) for manual recording of the sensor output. The minimal range of the voltage display was ±20 mV, at which setting the photodiode sensor could measure the irradiance up to 20 µW/cm² but measurements below 10 µW/cm² (i.e. 10 mV reading on the PicoScope) could be noisy.

2.2 Fiber-optical probe
A non-calibrated fiber-optical probe (IP159; Medlight SA, Ecublens, Switzerland) shown in Figure 1B was used for cross-examining the dynamic range of the irradiance as measured by the photodiode sensor. The fiber-optical probe had a spherical diffuser tip of 1.7 mm in diameter connected to a 3-m long fiber of 400 µm core diameter that was terminated to an SMA-905 connector. This fiber-optical probe had a ± 10% isotropy in air over a wavelength range of 480–800 nm. The fiber-optical probe output was measured by one of two spectrometers upon the availability. A compact UV/VIS/NIR (300–1000 nm) spectrometer (NT58-303, Edmund Optics Inc., Barrington, NJ, USA) with a 16-bits intensity resolution and with SMA-905 connection [16] was used for the irradiance measurement in an aqueous tissue-simulating phantom. For the irradiance measurements in tissue, a spectrophotometry unit [17] with low dark count, high sensitivity, and flexible gain and exposure control was used. The spectrophotometry unit consisted of a spectrometer (SpectraPro 2300i; Acton Research, Trenton, NJ, USA) having three gratings with varying groove densities (the 150 grooves/mm grating was used for this study), and a gated-intensified charge-coupled device (CCD) camera (PI-MAX, Acton Research, Trenton, NJ, USA) with a 16-bit intensity resolution. The fiber-optical probe was coupled to the spectrometer entrance slit via an in-house developed fiber adaptor.

![Photodiode sensor](A) ![Fiber-optical probe](B)

Figure 1: Sensors used for measuring the irradiance under transcutaneous laser application. (A) A miniature photodiode sensor is packaged in a glass enclosure with a dimension of 14 mm × 5 mm × 3 mm. (B) A fiber-optical probe has a spherical diffuser tip of 1.7 mm in diameter connected to a 400-µm core fiber terminated to an SMA-905 connector.
2.3 Laser and treatment head
A laser unit (Companion CTS Therapy System; LiteCure LLC, Newark, DE, USA) routinely used for animal patients in the Veterinary Medical Teaching Hospital of the Center for Veterinary Health Sciences at Oklahoma State University was used for this study. The CTS unit is a class-IV solid state laser running at CW or pulsed mode at 980 nm or 810 nm, with an output power adjustable between 0.5 and 15 W at an increment of 0.5 W. This CTS laser unit has several laser delivery heads with different beam sizes. Among these treatment heads, a small-cone treatment head emits the narrowest and collimated beam (with a 1-cm diameter), thus delivering the highest power density. This small-cone treatment head was used for this study, as its relatively simple beam profile made it possible to use the irradiance within an aqueous tissue-simulating phantom measured by the calibrated photodiode sensor to evaluate model-predictability based on a realistic beam profile. A pilot beam of 3.5 mW at 650 nm is also emitted via this small-cone treatment head.

2.1.4 Animal preparation
The animal protocol for this study involving a cadaverous dog was reviewed and exempted by the Institutional Animal Care and Use Committee of Oklahoma State University. The use of cadaverous tissue is in line with the Article 18 (Sharing organs and tissues) of the EU DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes: Member States shall facilitate, where appropriate, the establishment of programmes for the sharing of organs and tissues of animals killed [18]. One cadaverous dog of 18 kg weight was acquired for this study from a regional animal shelter after it was euthanized due to a terminal condition not affecting the dermis, musculature, bones or nervous system as part of the normal animal control protocol for that shelter.

Two sets of tissue measurements were conducted with this dog, respectively at three weeks apart. Since a frozen cadaver was received, the dog was allowed to thaw to room temperature for 24 h prior to each of the two days for tissue measurements. The first set of tissue studies included intra-cranial measurement via craniotomy and intra-spinal measurement at only the T13–L1 level via hemilaminectomy, with up to 10 W power delivered by the small-cone laser treatment head on a closely clipped (Oster A5 Cordless Clipper / #40 clipper blade / Valley Vet Supply, Marysville, KS, USA) area of skin 1 cm from the dorsal cranial and dorsal lumbar skin surfaces. The second set of tissue studies included skin-to-muscle and skin-to-spine transmission measurements at positions corresponding to T12–13, T13–L1, L1–2, L2–3, and L3–4 regions.

The skin-to-muscle transmission was measured by placing the photodiode sensor under the muscle and in direct contact with the vertebra, and applying 1 W surface power via the small-cone treatment head at 1 cm away from the prepared skin. The skin-to-spine transmission was measured by placing the photodiode sensor in the spinal canal, and applying 10 W surface power via the small-cone treatment head at 1 cm away from the prepared skin. In all tissue measurements, the position of the treatment head was handheld stabilized to reach maximal readings from the sensors, and five readings were taken at each position. For the hemilaminectomy at T13–L1 on the right side of the vertebral column, the epaxial muscles were elevated from the dorsal spinous processes and lamina of T12–L2. A pneumatic drill and burr (3M A200 Minos, Surgical Power, Warsaw, IN, USA) were used to create an oval laminectomy from immediately caudal to the T12–13 facet to immediately cranial to the L1–2 facet and from the base of the dorsal spinous process to the laminar-body junction. The margins of the laminectomy were completed with rongeurs (Kerrison Rongeur, V. Mueller, CareFusion, San Diego, CA USA) to allow for access to the spinal canal. Similar procedures were sequentially extended for hemilaminectomy at T13–L1, L1–2, L2–3, and L3–4 on the right side of the vertebral column, on the day of the second study. The craniotomy was performed using the same pneumatic drill and burr as for laminectomy to create a 1.5 cm × 1.5 cm opening over the right parietal bone lateral to the sagittal crest. The segment of bone created during the craniotomy was removed for instrument placement and replaced, along with the dura mater, for testing.

2.5 Measurement protocol
In all tissue measurements, the photodiode sensor alone or alternatively the fiber-optical probe was placed in the position of interest. Placing the two sensors together for tissue measurements was considered, but was not implemented due to the difficulty of maintaining consistent relative positions between the two sensors at all sites of measurement and with good tissue contact. The sensor was inserted to the spinal canal or the cranial cavity and placed in contact with the dorsal-lateral aspect of the cavity. The contact of the sensor with the vertebra or the immediate adjacent bone was maintained by filling the cavity space with adjacent tissue, closing the soft-tissue with monofilament suture (PDS II, 3-0, 3-0, 3-0, 3-0, 3-0).
Ethicon, Somerville, NJ, USA) and filling any gap with 1% intralipid diluted from Intralipid® 20% (A 20% I.V. Fat Emulsion) Pharmacy Bulk Package (Baxter Healthcare Corporation, Clintec Nutrition Division, Deerfield, IL, USA) to remove air that could specular-deflect the light when propagating to the sensor. Surface laser power of 0.5–10 W at 980 nm was applied using the small-cone treatment head at 1 cm from the skin surface. The photodiode sensor has an irradiance responsivity of 0.8 V per 1 mW/cm² (or 0.8 mV per 1 µW/cm²) at 980 nm. The output by the fiber-optical probe corresponded to the peak value measured by the PI-MAX CCD mounted on the SpectraPro 2300i spectrometer at an arbitrary setting of gain and exposure time.

The skin-to-muscle penetrations over the T13–L1 to L3–4 levels were measured by embedding the photodiode sensor between the soft tissue and the vertebra. A stainless steel 22 G × 1½” needle (Terumo, Elkton, MD, USA) was introduced percutaneously with the needle tip at the targeted region for the sensor placement, to be used as a radio-opaque marker for localization of the sensor under ultrasound imaging (see Figure 2). The needle placement was necessary for identifying the photodiode sensor against the echoic artifacts caused by the adjacent vertebra, and maintaining the lateral position of the sensor with respect to the vertebra segment of interest, when the sensor was covered by the soft tissue. Comparatively, the sensor positions after intra-spinal placement were relatively easily maintained. An ultrasound console (ProSound Alpha 6; Hitachi Aloka Medical America, Wallingford, CT, USA) together with a 9120 transducer at 8 MHz was used for ultrasound visualization of the needle placement and identification of the adjacent photodiode sensor, with a sectional field-of-view of radius 3.5 cm, a gain setting of 75, and a contrast setting of 10. The skin-to-spine penetrations over the T13–L1 to L3–4 regions were measured by placing the photodiode sensor alone in the spinal canal.

![Figure 2](image_url): A stainless steel 22 G × 1½” needle was introduced percutaneously (left) in adjacent to the photodiode sensor embedded between the muscle and the vertebra as a marker on ultrasound image (right) for assessing the position of the sensor.

3 RESULTS

3.1 Transcutaneous transmission to the cranial cavity under a surface irradiation

Transmission to the cranial cavity under a surface irradiation of 980 nm light at 0.5–10 W as measured by both the photodiode sensor and the fiber-optical probe is shown in Figure 3A. The output of the photodiode sensor was converted according to its sensitivity at 980 nm, i.e., 0.8 V per 1 mW/cm² irradiance, the same as its sensitivity at 810 nm as used for the measurements in the aqueous tissue simulating phantoms for aligning the model predictions. In Figure 3A, the average of the five absolute readings from the photodiode sensor at each irradiance level was displayed, but all five readings from the fiber-optical probe at each irradiance level were displayed with a peak value at the 10-W irradiance normalized to that of the averaged value of the photodiode sensor for comparing the changes of the two sets of sensor outputs as a function of the surface power. At 10 W surface power, an intra-cranial irradiance of 145 µW/cm² was measured by the photodiode sensor, and at a surface power lower than 10 W, the readings from the photodiode sensor...
indicated a decrease of the intra-cranial irradiance in proportion to the surface power. The readings from the fiber-optical probe over the 5–10 W range of the surface power varied significantly, and the range of the variance was shown to be roughly in proportion to the surface power. The readings from the photodiode sensor, when not averaged, varied much less than those from the fiber-optical probe, only because estimating the photodiode-sensor output on the PC-based oscilloscope was not as responsive as reading the fiber-optical probe output on the spectrophotometer interface.

### 3.2 Transcutaneous transmission to the T13–L1 level of the spinal canal under a surface irradiation

Transmission to the T13–L1 region of the spinal canal under a surface irradiation of 980 nm light at 0.5–10 W as measured by both the photodiode sensor and the fiber-optical probe is shown in Figure 3B. At 10 W surface power, an intra-cranial irradiance of 130 µW/cm² was measured by the photodiode sensor, and at a surface power lower than 10 W, the readings from the photodiode sensor indicated a decrease of the intra-spinal irradiance in proportion to the surface power. The readings from the fiber-optical probe over the 5–10 W range of the surface power varied significantly, and the range of the variance was shown to be roughly the same over the surface power range of 3–10 W.

![Photodiode sensor vs Fiber-optical probe](image1.png)

**Figure 3:** Transcutaneous transmission to the cranial cavity (A) and to the T13–L1 region of the spinal canal (B) under a surface irradiation of 980-nm light at 0.5–10 W as measured by both photodiode sensor and the fiber-optical probe. For either the photodiode sensor or the fiber-optical probe, and at each power setting of the surface irradiation, five readings from the sensor or the probe were taken, with the average of the five absolute readings displayed for the photodiode sensor.

![Sensor output vs Laser power](image2.png)

**Figure 4:** Comparison of transcranial transmission and transcutaneous transmission to the T13–L1 region of the spinal canal under a surface irradiation of 980-nm light at 0.5–10 W as measured by the photodiode sensor. Each data point at each power setting of the surface irradiation represents an average of five readings from the sensor.
For a more direct comparison of the irradiance reaching the spinal canal and transcranial delivery under the same level of surface irradiation, the measurements by the photodiode sensor in Figure 3A and B are combined in Figure 4. Over the range of 5–10 W surface power, the intra-cranial delivery was slightly lower than transcranial delivery, with an irradiance of 130 µW/cm² at the T13–L1 level compared to an irradiance of 145 µW/cm² at the cranial cavity under the same 10-W surface power. At a surface power less than 4 W, the readings of the photodiode sensor were at the level of 50 mV or less, which was not reliably estimated on the PC-based oscilloscope. As a result, the readings of the local irradiance in the spinal canal and the cranial cavity at a surface power less than 4 W were indifferent. Overall, transcutaneous delivery of a 10-W surface power over a 1-cm diameter beam to the cranial cavity and the shallowest region of the spinal canal were comparable to each other.

### 3.3 Skin-to-muscle and skin-to-spine transmission at T13–L1 to L3–4 regions of the spinal canal under a surface irradiation

Skin-to-muscle and skin-to-spine transmissions at the T12–13, T13–L1, L1–2, L2–3 and L3–4 levels of the spine under a surface irradiance of 980-nm light measured by the photodiode sensor only are shown in Figure 5. A 1-W surface power was applied for the skin-to-muscle transmission measurements, and a 10-W surface power was applied for the skin-to-spine transmission measurements, at each of the five regions. In Figure 7, the positions of the photodiode sensor are indicated on an independent fluoroscopy image obtained previously from a different dog [16] for illustration purpose only.

![Figure 5: Skin-to-muscle and skin-to-spine transmissions under a surface irradiation of 980-nm light measured by the photodiode sensor only. (A and C): When measured without the vertebra attenuation and under 1-W surface irradiation, the transmitted light was measurable at an irradiance-response scale of mW/cm² at the T12–T13 and T13–L1 regions but saturated at L1, L2, L3 regions. (B and D): When measured with the vertebra attenuation and under 10-W surface irradiation, i.e., 10× of that used for (A) and (C), the transmitted light was measurable at the T12–T13 and T13–L1 regions but at the noise floor at L1, L2, L3 regions, even though the irradiance-response scale was significantly more sensitive at µW/cm², i.e., 1/1000 of that for (A) and (C).](image-url)
As shown in Figure 5, under a 1-W surface power, an irradiance of 7–8.7 mW/cm² transmitted from the skin through the muscle at the T12–13 and T13–L1 levels, but the skin-to-muscle penetrations at L1–2, L2–3, and L3–4 levels saturated the photodiode sensor (at 9.2 mW/cm²). Comparatively, under a 10-W surface power, an irradiance at the level of 33 µW/cm² transmitted from the skin through the spine at the T12–13 and T13–L1 regions, but the skin-to-spine penetrations at L1–2, L2–3, and L3–4 regions were noisy at the level of 10 µW/cm². For the measurements at T12–13 and T13–L1, the skin-to-muscle transmission at 1-W surface power and the skin-to-spine transmission at 10-W surface power when combined translate to an approximate 0.05% (1/2000) transmission by the bone alone at those two regions of the spine. For the measurements at L1–2, L2–3, and L3–4, the skin-to-muscle transmission at 1-W surface power saturating the sensor and the skin-to-spine transmission at 10-W surface power at the noise floor, can be interpreted to an approximate 5-times of less transmission in comparison to the T12–13 and T13–L1 regions, totaling 0.01% (10⁻⁴) transmission by the bone alone at the three regions of the spine.

4 DISCUSSION

It is of note that the skin-to-spine transmission at the T13–L1 level measured during the second set of tissue studies for intra-subject comparison of transmissions to the five regions of spinal canal was approximately 1/4 of that measured during the first set of tissue studies for intra-subject comparison of transcranial and transcutaneous delivery to spine under the same 10-W surface power. The reduction of the skin-to-spine transmission at the same perceived segment of the spinal canal might seem to be significant; however, it could indeed be a small portion comparing to the level of the total attenuation estimated for that region of the spine. The four-times of reduction of the transmission at the T13–L1 region of the spine, assuming a 4-cm homogeneous tissue to penetrate, is equivalent to a 0.346 cm⁻¹ increase in the effective attenuation coefficient. This change of 0.346 cm⁻¹ in effective attenuation coefficient corresponds to a 15.6% increase over a baseline of 2.22 cm⁻¹, if the system of skin-to-spine tissue at the T13–L1 region is similar to the system of human head [2]. A speculated level of 15.6% stronger effective attenuation of tissue could be primarily caused by tissue degradation during the freezing-thawing process occurred between the two days of tissue study that were 3-weeks apart. McElderry et al. [19] have recommended freezing and thawing bone tissue only once to maintain accurate results for Raman spectroscopy, because degradation of bone tissue after repeated freezing and thawing had a significant impact on both matrix and mineral spectroscopic features and may interfere with bone quality measurements as early as one freezing.

The first set of study showed that transcutaneous transmission to the T13–L1 level of the spinal canal was comparable to transcranial transmission in the same subject measured under similarly controlled experimental conditions. The 10-W surface power illuminating as a collimated beam normal to the skin surface was expected to experience only a 2% specular reflection, therefore 98% of the 10 W surface power were transmitted into the tissue to result in a 12.5 W/cm² entrance irradiance in tissue. The 0.145 mW/cm² and a 0.13 mW/cm² irradiance measured at the cranial cavity and the T13–L1 region of the spinal canal, thus correspond to a 1.14 × 10⁻⁴ mW/cm² and 1.02 × 10⁻⁵ mW/cm² for an entrance irradiance of 1 mW/cm². These numbers are at the same level of the irradiance measured at approximately 5 cm depth below the scalp of human head for a 1 mW/cm² power density at the scalp [2].

Whether this level of transcutaneously transmitted irradiance reaching the spinal canal will be sufficient to induce bio-stimulatory effect in the spinal cord will be a subject of further investigation. In transcranial PBM applications by Tian et al. [20], a 1064-nm laser stimulation at the center and the right side of the forehead with a surface irradiance as small as 0.25 W/cm² has shown to induce an increase of oxygenated hemoglobin concentration and a decrease of deoxygenated hemoglobin concentration in both cerebral hemispheres. The 0.25 W/cm² corresponded to a surface laser power of 3.4 W applied over an area of 13.6 cm² or a beam of 4cm in diameter. At this surface irradiance, a 250 µW/cm² terminal irradiance can be reached at a 3-cm depth of the brain tissue with an effective attenuation coefficient of 2.22 cm⁻¹ [2]. Therefore, it can be articulated that a terminal irradiance of 100 µW/cm² in the spinal canal may be beneficial to inducing bio-stimulatory effects in the spinal cord; yet, the therapy outcomes of PBM may not be reached below a dose threshold. Anders et al. [21] determined the transcutaneous penetration of 980-nm wavelength light to the level of the peroneal nerve in vivo in White New Zealand rabbits, using a power density of 10 mW/cm² at the level of the peroneal nerve.
nerve over a beam area of 8 cm\(^2\) and a total energy of 65 J. \textit{In-vivo} penetration of the infrared light measured in anesthetized rabbits showed that on average, 2.45\% of the light applied to the skin reached the depth of the peroneal nerve, and the 2-W parameters significantly improved axonal regrowth.

The dose effective for PBM has to be delivered with a safe surface protocol not causing collateral thermal damage. In [20], transcranial stimulation was applied for 10 min at a surface irradiation of 0.25 W/cm\(^2\) and a total energy density of 150 J/cm\(^2\). Projecting this level of energy density to transcutaneous application for the potential of bio-stimulation in the spinal canal in the scale of a 18-kg dog as was tested in this present study, the total surface energy density of 150 J/cm\(^2\) was reached in just 12 s. Although the terminal irradiance or power density at the shallowest T13–L1 level under a surface power of 10 W may be comparable to that at the bio-stimulatory depth in head [20], the total energy density at the T13–L1 level under 12 s of 10 W surface power could reach only 1/50 of that at the bio-stimulatory depth in head under 600 s of 3.4 W surface power [32]. That translates to 50 times longer duration of the surface application at 10 W for reaching the same amount of energy density in the spinal canal as that reached at the bio-stimulatory depth in head [20]. In the present study it was observed that, the 10 W surface power delivered over a 1-cm collimated beam for more than 10 s could cause collateral thermal damage when applied to the prepared skin of the cadaverous dog. It thus remains a significant challenge to deliver a potentially bio-stimulatory energy density under a safe surface power density to even the shallowest region of the spinal canal, using the conventional treatment beam setting.

These preliminary results, limited to only one subject, indicate that light reaching the shallow region of the spinal canal of a cadaverous dog under a clinically safe surface power is comparable to the transcranial delivery. However, it is to acknowledge that a single-sensor configuration had not been desirable for accurate profiling of the transmitted irradiance along the spinal canal, as repositioning the sensor for measurements at multiple segments of the spinal canal compromised the consistency of measurement conditions. Accessing and assessing the light irradiance profile at multiple positions along the spinal canal under a narrow conventional beam or an elongated beam will be critical to evaluating if sufficient bio-stimulatory dose can be delivered to the spinal cord. Work is ongoing to develop a flexible sensor module consisting of multiple photodiode sensors for multi-site measurements of the intra-spinal irradiance at consistent settings. Additionally, the effects of contact versus non-contact beam delivery and prepared versus unprepared skin surface are among the conditions to be evaluated in future studies. Another limitation of this study was the lack of hemoglobin variation over the beam-interrogated tissue area which may introduce additional dosimetry confounder for \textit{in-vivo} investigations.

5 CONCLUSIONS

A hemilaminectomy and a craniotomy were performed on a 18-kg cadaverous dog to assess transcutaneous delivery of the irradiance to the shallowest region (T13–L1) of the spinal canal in comparison to transcranial delivery. Additionally, a hemilaminectomy for exposing the T12–13, T13–L1, L1–2, L2–3, and L3–4 segments of the spinal canal were performed to assess the skin-to-muscle penetration and skin-to-spine penetration at the corresponding positions under a 1-W or 10-W surface power. Transcutaneous delivery of the irradiance to the shallowest region (T13–L1) of the spinal canal seemed to be comparable to transcranial delivery. Transcutaneous delivery to other segments of the spinal canal that are either deep or bound with thicker vertebra is challenging, indicating a potentially narrow tissue window for transcutaneous bio-stimulation of the spinal cord.

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