Research Article

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# Assessment of the Microcirculation in Superficial and Deep Biotissue with Photobiomodulation

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## Abstract

***Background***

*Quantitative measurement of blood flow in specific biotissue targets is crucial for photobiomodulation therapy (PBMT). PBMT has been applied on many clinical applications. However, the conclusive confirmation of its effects on microcirculation and laser wavelength dependency in superficial and deep tissues is lacking.*

***Objective***

*This study examines the microcirculatory response and laser wavelength dependency of red and near infrared (NIR) lasers in biotissue. Laser Doppler flowmetry (LDF) was used to assess the flux and velocity of erythrocytes in buccal tissue when exposed to 660 nm and 830 nm laser radiation.*

***Discussion***

*The microcirculatory response of subjects during and after laser radiation were analyzed. Each laser has an energy density of 73.17 J/cm$^2$. Both the 660 nm and 830 nm lasers demonstrated the capability to enhance microcirculation in both superficial and deep tissues. Furthermore, we observed that laser wavelengths at 660 nm and 830 nm respectively enhanced the microcirculatory response in superficial and deep tissues. Importantly, this cumulative effect persisted for at least 10 minutes.*

***Conclusions***

*The present results indicate that PBM can effectively improve local blood perfusion in specific target areas and peripheral biotissues. These findings have potential applications in enhancing wound healing and local microcirculation within the target areas.*

**Keywords:** Photobiomodulation, Near Infrared, Laser Doppler Flowmetry, Erythrocytes, Microcirculation

## 1. Introduction

The role of microcirculatory blood flow in biological tissues is an important aspect of human physiological regulation [1, 2]. Adequate perfusion via the microcirculatory network is necessary to maintain normal hemodynamics in target and peripheral tissues. Several studies have investigated methods to improve tissue perfusion, such as far infrared radiation, thermal therapies, cupping, and photobiomodulation (PBM) [3-8]. PBM known as low-power laser (light) therapy, is a recommended non-invasive, painless, and safe biostimulation method. The cellular function of PBM has been attributed to the absorption of red and near infrared (NIR) light by the cellular respiratory chain [9, 10]. Short and long wavelengths are recommended for superficial and deep target tissues, respectively [11]. For instance, the efficacy of the 810 nm laser is significantly higher than that of the 660 nm laser in treating patients with trigeminal neuralgia and temporomandibular disorders [12].

PBM can be used for microcirculation improvement and increased diameter of arterial, venous, and lymphatic vessels [13-14]. The vessel diameter and blood flow of tissues following PBM have been examined [15–21]. Ihsan demonstrated that the 904 nm laser may enhance adenosine, growth hormone, fibroblast growth factor, fiber/capillary ratio, and capillary diameter in rabbits, therefore accelerating collateral circulation and improving microcirculation [15]. Recently, it was shown that the cerebral blood flow (CBF) response of mouse can be triggered by varying light intensities [16]. Maegawa and co-workers measured microvascular blood

flow using the dosage dependence of PBM [17]. The findings revealed that the 830 nm laser caused substantial alterations in rat arteriolar blood flow at 38.2 mW/mm$^2$ compared to 6.4 mW/mm$^2$. The biphasic response known as the "Arndt-Schulz Law" for PBM demonstrated that a low dose is helpful, while a greater amount is detrimental [22]. Thus, the appropriate wavelength [9–11] and dose [16, 17, 22] of PBM are critical for physiological control in biotissues.

Recently, the effect of mixture of three wavelengths (625, 660, and 850 nm) of light-emitting diode (LED) on chronic wounds was investigated in both in diabetic and non-diabetic patients by measuring blood flow with laser Doppler flowmetry (LDF) [7]. The results showed a significant increase in blood flow in the treated group of both diabetic and non-diabetic patients ($p = 0.040$ and $p = 0.033$). Our previous study also found that the palm with 660 nm LED radiated can increased the velocity of erythrocytes in the nailfold capillary by approximately 1.7-fold [18]. In another study, a 20% increase in CBF was found in a patient in a permanent vegetative state who received an 850 nm LED on the left anterior frontal lobe [19]. Gavish et al. demonstrated that 830 nm LED induced a 27% increase in microcirculatory flow, which increased to 54% during 20 minutes follow-up by LDF measurement [8]. In addition, both immediate and long-lasting arteriolar vasodilation can be induced. Based on Poiseuille's law, the volumetric flow rate can be influenced by the diameter of the blood vessel [20]. Therefore, the diameter of the blood vessel is an important factor in determining the blood flow.

Recently, the maximum dose and its distribution of the 830 nm laser in the deep tissue can be quantitatively calculated in our study [23, 24]. The results showed that the penetration depth can be achieved up to 1 cm, which can be used for deep target treatment applications. In contrast, the power density of the 660 nm laser is focused on the shallow tissue [23], which can be used for the superficial tissue repair process. 660 nm and 830 nm lasers as a safe modality regarding any cytotoxic and macroscopic have been mentioned by Logan [21] and Sasaki [25], respectively. However, the effect of microcirculation and laser wavelength dependence in superficial and deep tissues has not been conclusively confirmed. In the present study, the microcirculation and the wavelength dependence of 660 nm and 830 nm lasers in superficial and deep tissues by LDF were investigated.

## 2 Methods

### 2.1 Experimental Procedure

In this study, two case is presented in this study. The age of a male and a female is 20 and 21 years old, respectively. The buccal tissue was selected as the target area for evaluating the microcirculatory changes in both superficial and deep tissues using PBM. The thickness of the buccal tissue for the male and female subjects was 13.65 mm and 9.84 mm, respectively. For superficial tissue measurement, the optical probe of the LDF was positioned 15 mm away from the center of the laser output. For deep tissue measurement, another optical probe was placed opposite the laser source, as depicted in Figure 1(a). The study followed a three-stage protocol, as shown in Figure 1(b). First, the subjects were instructed to rest for 10 minutes, and baseline measurements of the flux and velocity of erythrocytes were recorded by LDF for 3 minutes. In the second stage, the buccal tissue of participant was radiated with 660 nm for 10 minutes, while the microcirculation of both superficial and deep tissues were simultaneously measured by LDF. In the third stage, the microcirculation was measured again for 10 minutes. After 1 week, the experimental procedure was repeated, and the 830 nm laser was used to radiated on the buccal tissue of participant. Each participant will receive two lasers in the study. Finally, the flux and velocity of erythrocytes were analyzed and compared to the baseline.

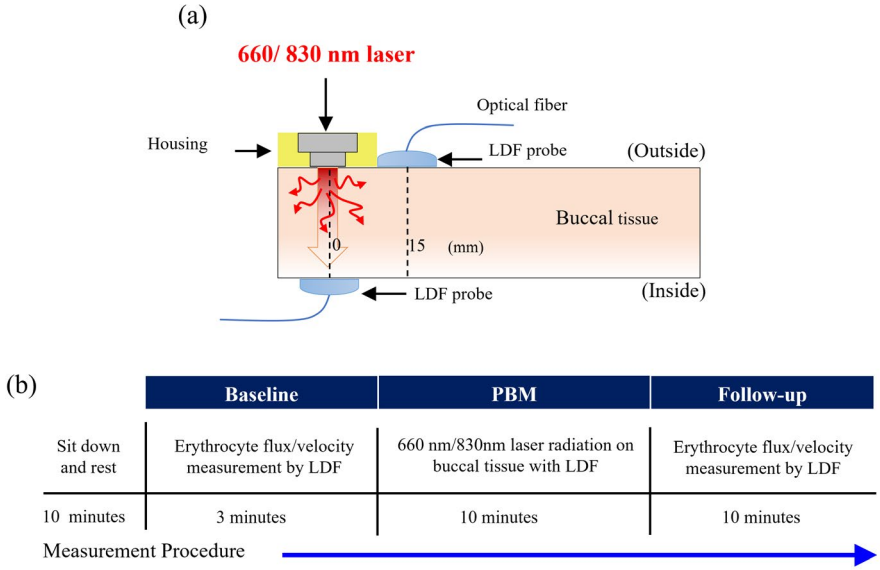


**Figure1:** (a) Schematic of the 660/830 nm laser radiated on the buccal tissue with LDF measurement. (b) The protocol of this study.

## 2.2 Laser Device

In this study, an auto-current control (ACC) circuit (Figure 2(a)) was designed and fabricated on a double-sided circuit board (Figure 1(b)) to drive the laser [23]. Two lasers were used: an aluminum gallium indium phosphide diode laser (model: U-LD-66A051Ap/Dp, Pocket Laser, Union Optronics Corp., Taoyuan, Taiwan) with a wavelength of 660 nm ± 5 nm, and an aluminum gallium arsenide diode laser (model: T8350, Pocket Laser, Opto Focus Co., Ltd., New Taipei City, Taiwan) with a wavelength of 830 ± 10 nm. Both lasers had the same output power of 30 mW, and stable output powers were ensured after appropriate warm-up periods. The lasers were used to irradiate the subject's buccal tissue for 10 minutes, with an energy density of 73.17 J/cm$^2$ for each laser mm$^2$.

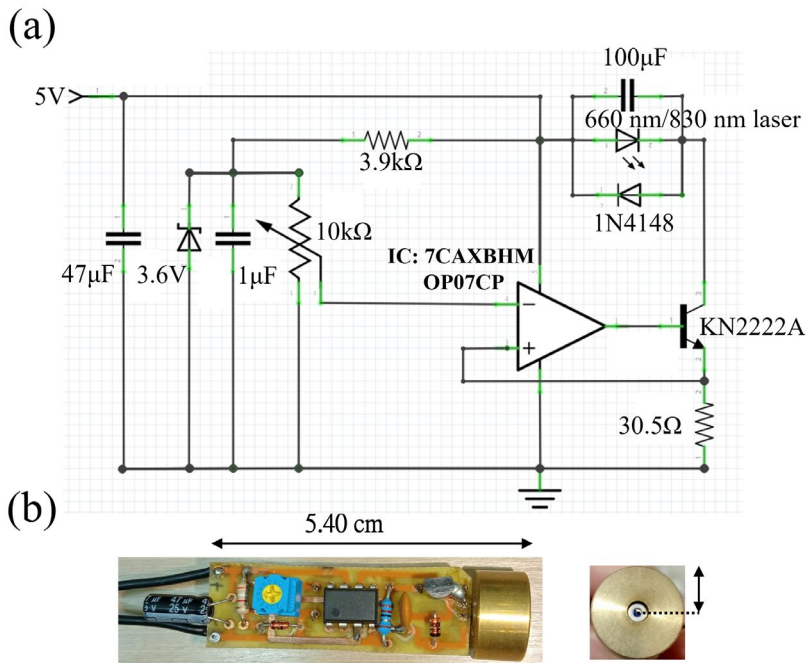


**Figure 2:** (a) Laser circuit design with auto-current control (ACC) [23] and (b) the laser device.

## 2.3 Laser Doppler Flowmetry

The study utilized a two-channel LDF instrument (model: moorVMS-LDF2; manufactured by Moor Instruments Ltd., United Kingdom) equipped with a 785 nm laser diode and temperature stabilization. The output power of the 785 nm laser was set at 2.5 mW. The laser was delivered through a flexible optical fiber to a sensor attached to the skin using a double-sided soft adhesive disk. When the laser penetrated the buccal tissue, it was scattered by moving red blood cells (RBCs). This led to a frequency shift between the incident and backscattered light from the RBCs, which was detected by photodetectors as the LDF signal, also known as the blood perfusion signal. This allowed for real-time assessment of microcirculatory changes in both superficial and deep buccal tissues. The flux and velocity of erythrocytes in these tissues with PBM could be quantitatively analyzed using perfusion units (PU) as the physical quantitative units.

## 3. Results

In the male participant, the flux of erythrocytes in both superficial and deep buccal tissues was found to increase with the use of the 660 nm laser (Figure 3(a)). Furthermore, the results revealed that the flux of erythrocytes in superficial tissue increased more rapidly compared to that in deep tissue upon exposure to the 660 nm laser. The flux of erythrocytes continued to increase even after the cessation of the 660 nm laser. Additionally, the velocity of erythrocytes in both superficial and deep tissues was observed to increase with the use of the 660 nm laser, which is consistent with the flux of erythrocytes in the tissues (Figure 3(b)).

In addition, the flux of erythrocytes in superficial and deep tissues can also be increased by the 830 nm laser. The results showed that the flux of erythrocytes in deep tissue can be rapidly increased by an 830 nm laser (Figure 3(c)). After the 830 nm laser cessation, the flux of erythrocytes can be continuously increased. Also, the velocity of erythrocytes in superficial and deep tissues can be increased by an 830 nm laser, which is consistent with the flux of erythrocytes in tissues (Figure 3(d)).

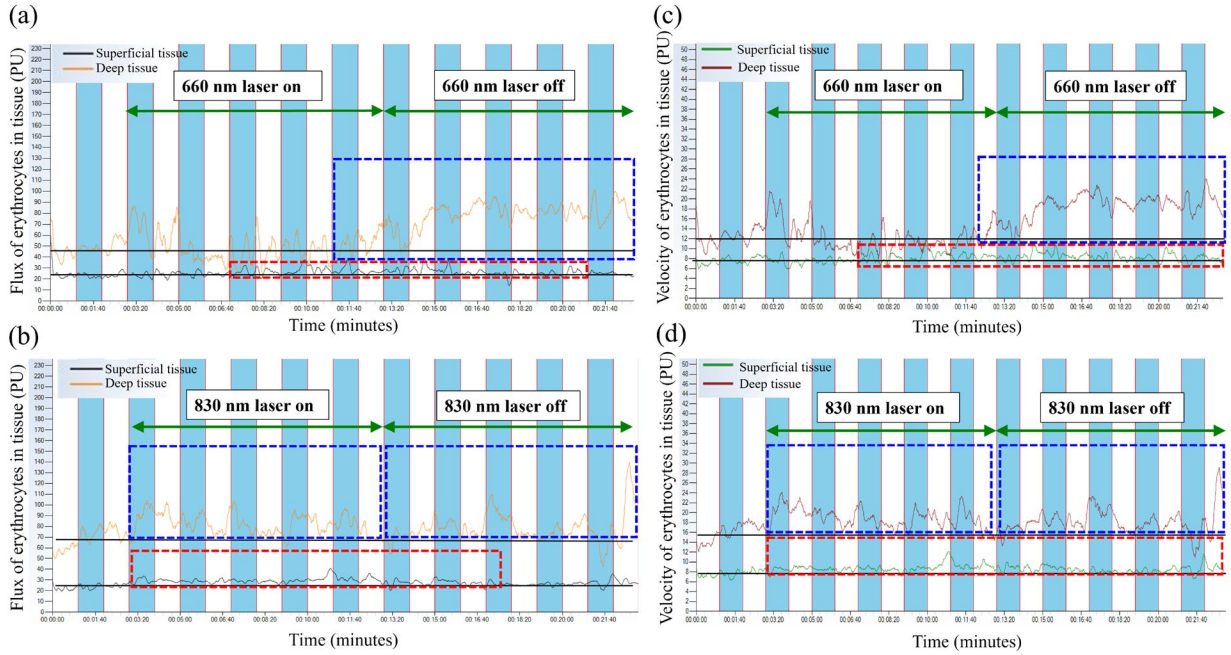


**Figure 3:** (a) The flux of erythrocytes in superficial/ deep buccal tissues with 660 nm and (b) 830 nm laser radiation. (c) The velocity of erythrocytes in superficial/ deep buccal tissues with 660 nm, and (d) 830 nm laser radiation for a male.

In the case of the female, the study analyzed the flux and velocity of erythrocytes in the same target tissues, as shown in Figure 4. Similarly to the male case, the flux and velocity of erythrocytes in superficial tissue were increased more rapidly than in deep tissue by the 660 nm laser (Figure 4(a) and (b)). Both the flux and velocity of erythrocytes showed a continuous increase after the cessation of the 660 nm laser. Additionally, the flux and velocity of erythrocytes in superficial and deep tissues were increased by the 830 nm laser. The flux and velocity of erythrocytes in deep tissue were increased more rapidly with an 830 nm laser than with 660 nm laser radiation (Figure 4(c) and (d)). After the 830 nm laser cessation, the flux of erythrocytes showed a continuous increase.

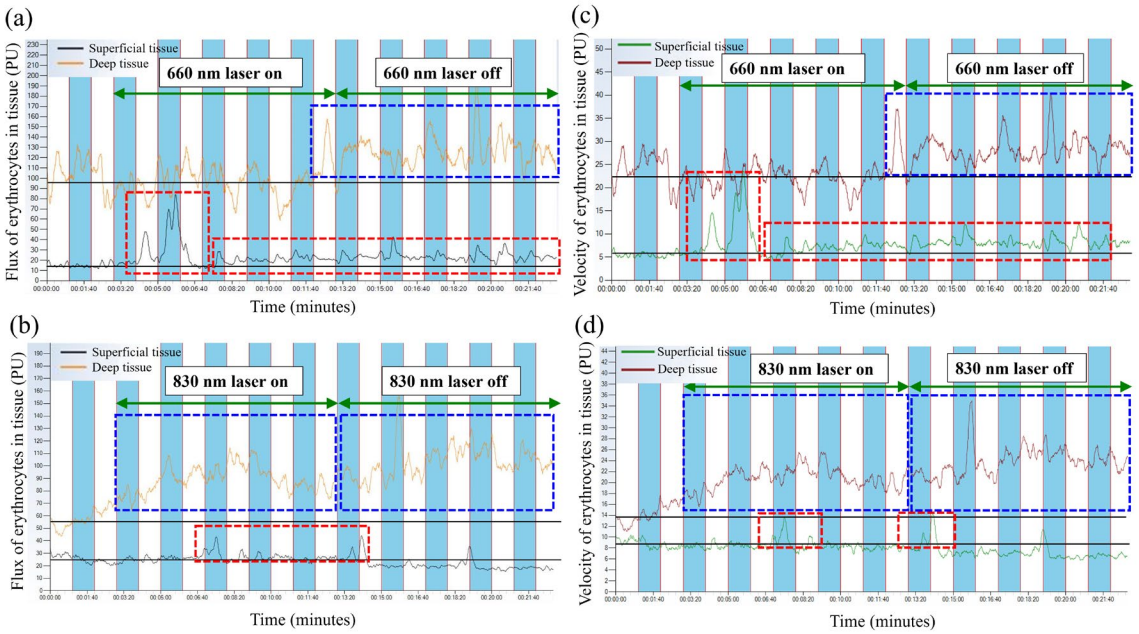


**Figure 4 (a):** The flux of erythrocytes in superficial/ deep buccal tissues with 660 nm and (b) 830 nm laser radiation. (c) The velocity of erythrocytes in superficial/ deep buccal tissues with 660 nm, and (d) 830 nm laser radiation for a female.

Additionally, the ratio of flux and velocity of erythrocytes in superficial and deep buccal tissues with a 660 nm laser were analyzed. The flux of erythrocytes in superficial tissue increased by 1.09 and 1.59-fold during 660 nm laser radiation for the male and female, respectively, as shown in Figure 5(a). These results were sustained for at least 10 minutes. However, the flux of erythrocytes in deep tissue did not significantly change during the 660 nm laser radiation (Figure 5(b)). After the 660 nm laser cessation, the flux of erythrocytes in deep tissue increased by 1.63 and 1.20-fold for the male and female, respectively.

Similarly, the velocity of erythrocytes in superficial tissue can be increased by 1.05 and 1.45-folds during the 660 nm laser radiation for the male and female, respectively (Figure 5(c)). Moreover, the velocity of erythrocytes in superficial tissue continued to increase after laser radiation. Meanwhile, the velocity of erythrocytes in deep tissue can be increased by 1.50 and 1.16-folds after laser radiation for the male and female, respectively (Figure 5(d)).

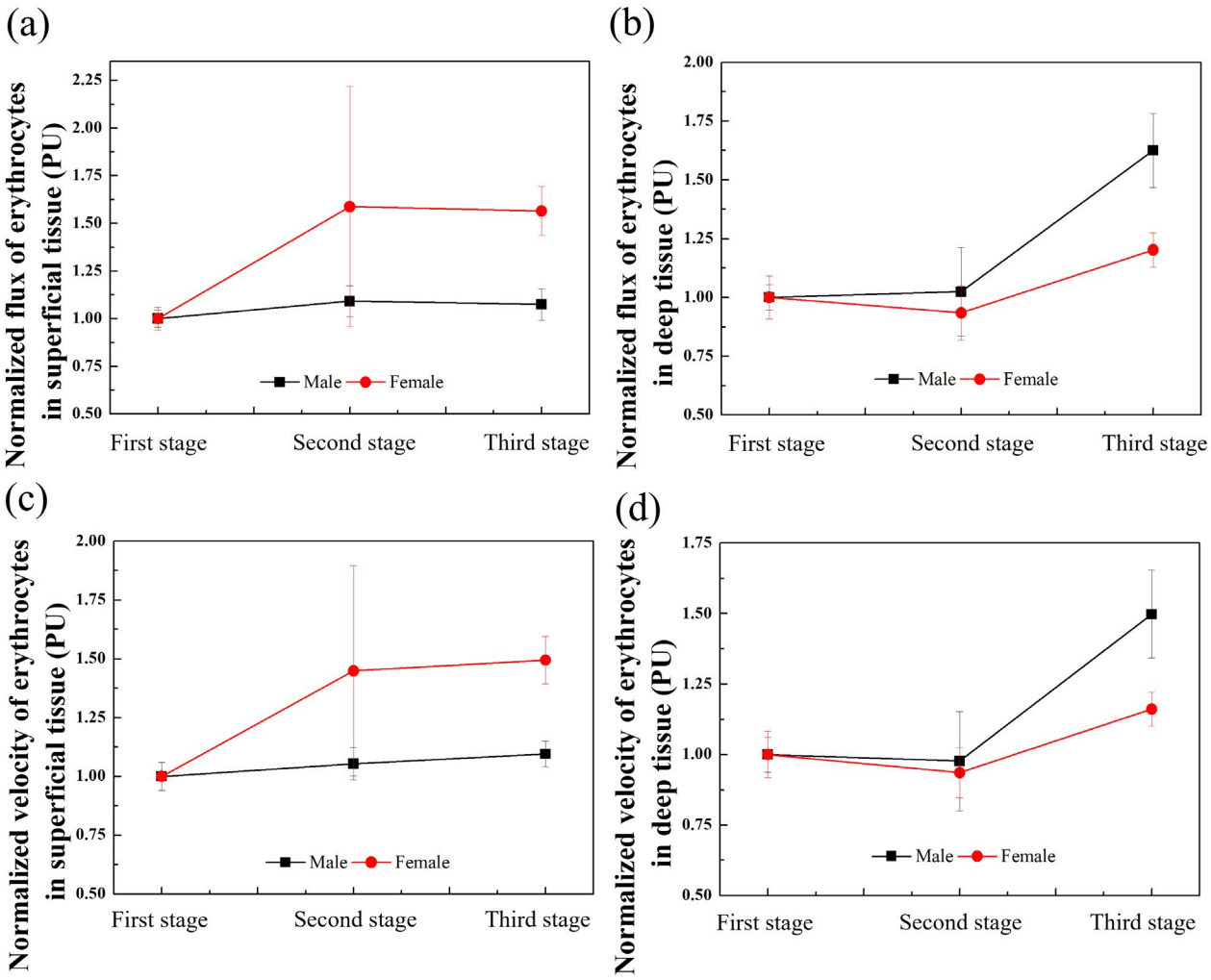


**Figure 5:** Normalized flux of erythrocytes in (a) superficial and (b) deep buccal tissues with a 660 nm laser. Normalized velocity of erythrocytes in (c) superficial and (d) deep buccal tissues with 660 nm laser (first stage: baseline; second stage: PBM radiation for 10 minutes; third stage: after the cessation of the PBM).

The ratio of erythrocyte flux and velocity in superficial and deep buccal tissues were analyzed using an 830 nm laser. The flux of erythrocytes in the superficial tissue increased by 1.25 and 1.05-fold for males and females, respectively, during 830 nm laser radiation (Figure 6(a)). However, the flux of erythrocytes in the superficial tissue decreased after the laser radiation. In the deep tissue, the flux of erythrocytes increased by 1.19 and 1.54-fold for males and females, respectively, during 830 nm laser radiation (Figure 6(b)). After the cessation of the 830 nm laser, the flux of erythrocytes increased by 1.14 and 1.79-fold for males and females, respectively, compared to the baseline.

During 830 nm laser radiation, the velocity of erythrocytes in the superficial tissue increased by 1.15-fold for males (Figure 6(c)). The results were maintained for 10 minutes; however, a slight decrease was observed in females. In deep tissue, the velocity of erythrocytes increased by 1.18 and 1.46-fold for males and females, respectively, during 830 nm laser radiation (Figure 6(d)). Furthermore, the velocity of erythrocytes continued to increase after laser radiation by 1.13-fold for males and 1.67-fold for females.

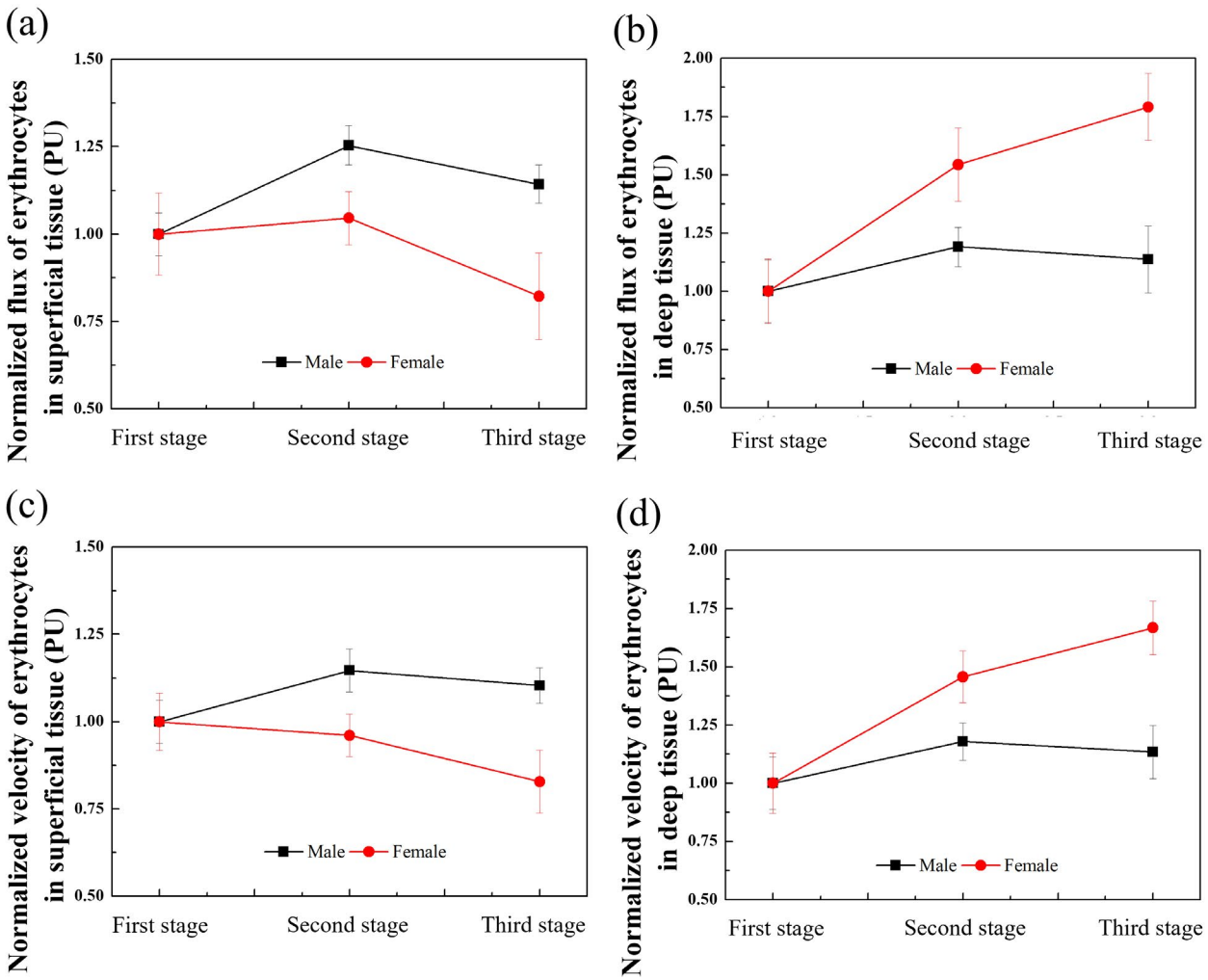


**Figure 6:** Normalized flux of erythrocytes in (a) superficial and (b) deep buccal tissues with the 830 nm laser. Normalized velocities of erythrocytes in (c) superficial and (d) deep buccal tissues with an 830 nm laser.

In this study, the ratio of flux and velocity of erythrocytes in superficial and deep tissues at the two wavelengths was compared between a male and a female (Table 1). According to the results, the flux and velocity of erythrocytes in superficial and deep tissues can be increased by 660 nm and 830 nm lasers in two cases. After PBM was stopped, the flux and velocity of erythrocytes in superficial and deep tissues could also be increased.

| Laser wavelength | Parameter of erythrocytes measurement (PU) | Male | | | Female | | |
|---|---|---|---|---|---|---|---|
| | | First stage | Second stage | Third stage | First stage | Second stage | Third stage |
| 660 nm | Flux of erythrocytes in superficial/ deep tissues | 1.00/1.00 | 1.09/1.02 | 1.07/1.63 | 1.00/1.00 | 1.59/0.93 | 1.56/1.20 |
| | Velocity of erythrocytes in superficial/ deep tissues | 1.00/1.00 | 1.05/0.98 | 1.10/1.50 | 1.00/1.00 | 1.45/0.94 | 1.49/1.16 |
| 830 nm | Flux of erythrocytes in superficial/ deep tissues | 1.00/1.00 | 1.25/1.19 | 1.14/1.14 | 1.00/1.00 | 1.05/1.54 | 0.82/1.79 |
| | Velocity of erythrocytes in superficial/ deep tissues | 1.00/1.00 | 1.15/1.18 | 1.10/1.13 | 1.00/1.00 | 0.96/1.46 | 0.83/1.67 |

**Table 1: the ratio of flux and velocity of erythrocytes in tissues for a male and a female with two lasers**

## Discussion

The action spectral wavelength of PBM ranges from 600 nm to 850 nm, influencing physiological responses [10] and tissue penetration depth [11], potentially making it useful in medical applications [12–15, 19, 29, 30]. In the present study, when a 660 nm laser was applied to buccal tissue, the ratio of flux (male: 1.09; female: 1.59) and velocity (male: 1.05; female: 1.45) increased more rapidly in superficial tissue than in deep tissue (Figure 5). Conversely, with the 830 nm laser, the ratio of flux (male: 1.19; female: 1.54) and velocity (male: 1.18; female: 1.46) increased more significantly in deep tissue (Figure 6). These results indicate that blood flow and velocity in superficial and deeper tissues can be quickly induced by 660 nm and 830 nm laser irradiation on the skin, respectively. These findings are significant as they have potential therapeutic applications targeting specific tissues. This suggests that improving blood perfusion with PBM could enhance the repair of target or peripheral tissues.

The present study's results were consistent with our recent study [23], which demonstrated that the penetration depth of an 830 nm laser was greater than that of a 660 nm laser in real tissue. Even after the laser was turned off, microcirculation continued to increase. Karu et al. noted that the cumulative effect could be observed when the target was irradiated with a laser [31]. In our study, we observed that the cumulative effect was sustained for at least 10 minutes, which was also consistent with our previous study [32]. In that study, the meridian values of patients with low back pain in the active group decreased on the first day but returned to their original values after 808 nm laser irradiation on the fifth day, possibly due to the accumulated dose of laser.

In PBM, the mechanism of photoresponse is attributed to the action spectral wavelength of the cellular respiratory chain, which is primarily situated in the mitochondria. Cytochrome c oxidase (CCO), an important chromophore, is the photoacceptor of the respiratory chain and is located in the mitochondria [9]. The pathway of PBM has been proposed to involve the alteration of mitochondrial intermembrane potential, the dissociation of nitric oxide (NO) from its binding site on CCO, the modulation of reactive oxygen species, the increase in adenosine triphosphate (ATP) production, and the induction of transcription factors [33, 34]. The photoresponse process involves the absorption of light by a photoreceptor, leading to signal transduction and a chain of molecular implications. Thus, PBM can induce cellular biological responses. The wavelengths of 660 nm and 830 nm are located in the therapeutic window [9, 10] and are attributed to the action and absorption spectra of CCO activity and ATP synthesized content [35]. Numerous studies have implicated ATP as a potent vasodilator in the regulation of vascular perfusion [36–39]. Dietrich and co-workers observed that vasodilation caused by the efflux of ATP from erythrocytes [36]. McCullough and colleagues demonstrated that local vasodilation was occurred along the arteriole when ATP was injected into arterioles [37]. In other studies, vasodilation of the arteriole can be found by injecting ATP into postcapillary venules [38] and larger venules [39]. Besides, ATP is involved in the purinergic control of vascular tone. P2X-purinoceptors and P2Y-purinoceptors are mainly located on vascular smooth muscle and the vascular endothelium, respectively [40, 41]. They play a role as mediators of vasoconstriction and vascular relaxation.

On the other hand, NO is another factor that causes vasodilation by PBM. Recently, NO release by 670 nm and 830 nm LEDs was found to cause vasodilation of mouse arteries [42]. Uozumi and co-workers demonstrated that NO was involved in increasing CBF by NIR laser radiation [43]. Besides, photo-induced relaxation of bovine mesenteric arteries is associated with a rapid increase in the cyclic guanosine monophosphate (cGMP) levels [44]. cGMP, as a mediator of vasodilation, and NO derived from the nitrovasodilators, result in increased cGMP synthesis [45]. These studies strongly suggest that ATP and NO play important roles in vasodilation and the increase in blood flow after PBM. Our study proposed a quantitative analysis method for PBM in biotissue to understand the microcirculatory response and the wavelength dependence of red and NIR light. The results can be used to

improve the microcirculation in superficial and deep tissues, which could be helpful for patients with chronic diseases, such as diabetes and chronic kidney disease.

This study also had several limitations. First, a small sample is enrolled in this study, so the results need to be verified by more people. Second, this study included healthy subjects, thus the present results may be different for the patients who have chronic diseases. Third, the proposed experimental tissue is the thickness of the buccal tissue. It means that we cannot measure the real flux and velocity of erythrocytes in deeper tissue than buccal tissue.

## Conclusion

In this study, the microcirculatory response and wavelength dependence of 660 nm and 830 nm lasers in buccal tissue were investigated. The results suggest that 660 nm and 830 nm lasers can rapidly enhance microcirculation in superficial and deep buccal tissues, respectively. Furthermore, the cumulative effect was observed to be sustained for at least 10 minutes. These preliminary findings demonstrate the efficacy of Photobiomodulation (PBM) in improving microcirculation in both superficial and deep tissues. However, it is important to note that this study had certain limitations, notably a small sample size. Future research endeavors with larger sample sizes and rigorous clinical trial analyses are essential to confirm these results and establish their generalizability.

**Conflict of Interest Statement** The author declares that they have no conflict of interest.

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**Author Contributions** Conceptualization and methodology, C.T. Su; investigation and validation, Y.T. Huang; writing - original draft preparation, review and editing, C.T. Su. All authors have read and agreed to the published version of the manuscript.

**Data Availability Statement** Data used to support the findings of this study are included in the article.

**Statement of Ethics** Ethical approval was not required for this study. Written informed consent was obtained for the publication.

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