Non-laser approach to photochemical tissue bonding

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Lasers have been used in the past to activate chemical cross-linking agents in photochemical tissue bonding; however, lasers are expensive and bound to only one wavelength, so that they cannot be used with bonding agents that have different activation wavelengths. Since light activation does not rely on the coherence of the radiation, but only on the wavelength and energy density, it is possible to use light sources other than lasers to generate this activation energy. In this paper the effectiveness of three bonding agents (methylene blue, rose bengal and fluoroscein) on human skin is tested using a non-laser light source. Tension and skin temperature measurements showed that skin adherence is as good as previously published laser-irradiated experiments, and that the light source does not induce thermal-related skin damage.

Keywords: Photochemical tissue bonding; non-laser light source; wound healing.

A pesar de ser costosos y estar sujetos a una única longitud de onda, los láseres son comúnmente utilizados para activar fotoquímicamente agentes utilizados en el proceso de unión de tejidos biológicos, conocido como unión tisular fotoquímica, con la desventaja de no poder ser utilizados con diferentes agentes químicos ya que éstos generalmente poseen diferentes longitudes de onda de activación. Debido a que la activación fotoquímica no depende de la coherencia de la radiación sino únicamente de la longitud de onda y la densidad de energía, entonces es posible utilizar otras fuentes de luz diferentes al láser para generar esta energía de activación. En este trabajo se analiza la efectividad de tres agentes utilizados en la unión tisular fotoquímica (azul de metileno, rosa de bengala y fluorosceina) en piel humana utilizando una fuente de luz diferente al láser. Se realizaron mediciones de tensión y temperatura de la piel que demuestran que la adherencia de la piel es tan buena como la obtenida con radiación láser y que esta fuente de luz alterna no induce daño térmico a los pacientes.

Descriptores: Unión tisular fotoquímica; sutura óptica.

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1. Introduction

The conventional methods for tissue repair use sutures, staples or clips. Sutures are favored because they are cost-effective, reliable and suitable for almost any type of tissue. However, due to their mechanical intrusion, the use of any of these conventional fasteners causes injury and they result in a foreign body being left in the tissue [1].

The feasibility of laser tissue welding was first shown by Yahr and Strully in 1964, when they used a neodymium laser to join small blood vessels [2]. In 1979, Jain and Gorisch reported the first reproducible experimental use of laser tissue welding [3].

While laser tissue welding is unlikely to replace sutures in all applications, it has been shown to achieve functionality comparable to that of conventional suturing techniques, with the added advantage of a reduction in operation times, skill requirements, suture and needle trauma, foreign-body reaction, and bleeding. The disadvantage of this procedure is the low strength of the resulting anastomosis and the thermal damage of tissue by direct laser heating and heat transfer [1].

Photochemical tissue bonding (PTB) has been investigated as an alternative method for tissue repair without the use of heat and its associated tissue damage. The technique uses chemical cross-linking agents that, when light-activated, produce covalent cross-links between the collagen fibers contained within the tissue [1]. PTB involves application of a dye to the walls of the wound, followed by laser irradiation. Immediate bonding results from absorption of light energy by the dye, which then initiates chemical reactions between proteins on the apposed tissue surfaces to form covalent crosslinks [4].

Lasers have been used in the past to activate chemical cross-linking agents in photochemical tissue bonding; however, lasers are expensive and bound to only one wavelength and so they cannot be used with different bonding agents, which usually have a different activation wavelength. Due to the fact that light activation does not rely on the coherence of the radiation but only on the wavelength and energy density, it is possible to use light sources other than lasers to generate the activation energy [5].

In this paper a non-laser approach to photochemical tissue bonding applied to three different dyes is investigated.

2. Materials and methods

A 50W halogen lamp and a set of optical filters were used as the activation light source. Three different photochemical bonding agents were evaluated, rose bengal (RB), methylene blue (MB) and flouroscein (Fl). For each of these bonding agents, an optical filter consistent with the maximum absorption spectrum of the chemical was used. The absorption spectra for the photochemical agents were measured using

an Ocean Optics USB4000 spectrometer that has a 200-1100 nm spectral range. The measured absorption spectra showed peak absorption at 490, 664 and 550nm for the fluoroscein, methylene blue and rose Bengal, respectively, which is consistent with previously published measurements [1]. Green, red and yellow optical filters were chosen to match the absorption spectra of the photochemical bonding agents. Figures 1-3 show the absorption spectra of the photochemical agents used and the optical spectra of the halogen lamp and optical filter combination.

Human skin was used to test the effectiveness of the three different photochemical bonding agents and the light source. The skin used in this study was considered healthy, obtained from elective surgeries, mainly lipectomies, provided by the surgery department of the "Dr. Ignacio Morones Prieto" hospital, and after its extirpation the skin was covered with sterile gauzes, impregnated with physiological solution (NaCl 9%) and kept under cold ischemia (2 to 5°C) for no more than 8 hours, during which time the bonding procedure was performed.

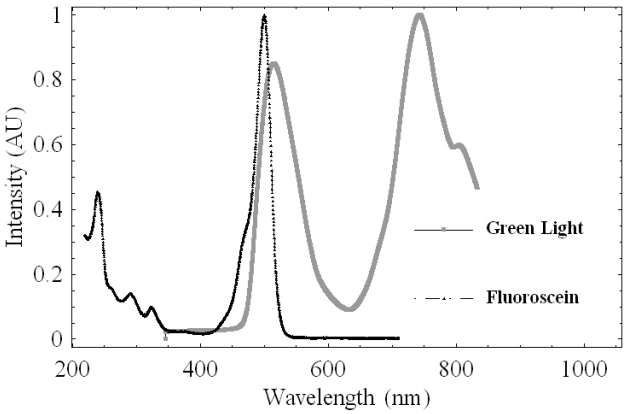

FIGURE 1. Absorption spectrum of floroscein and optical spectrum of the light source used for this bonding agent.

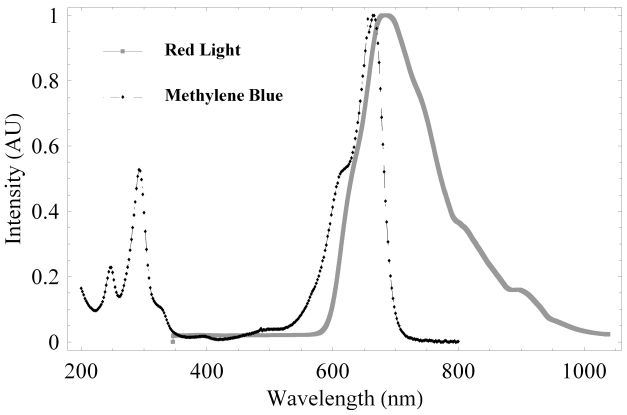

FIGURE 2. Absorption spectrum of methylene blue and optical spectrum of the light source used for this bonding agent.

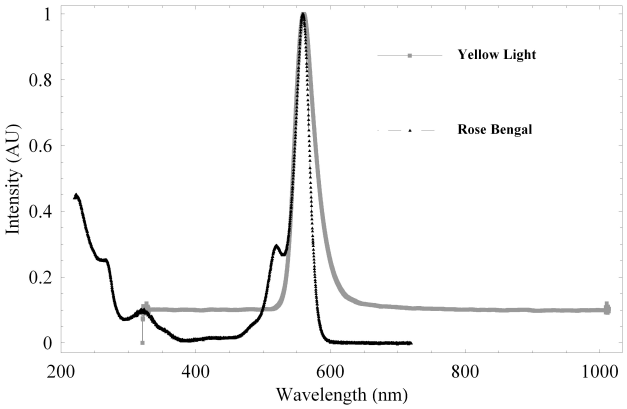

FIGURE 3. Absorption spectrum of rose bengal and optical spectrum of the light source used for this bonding agent.

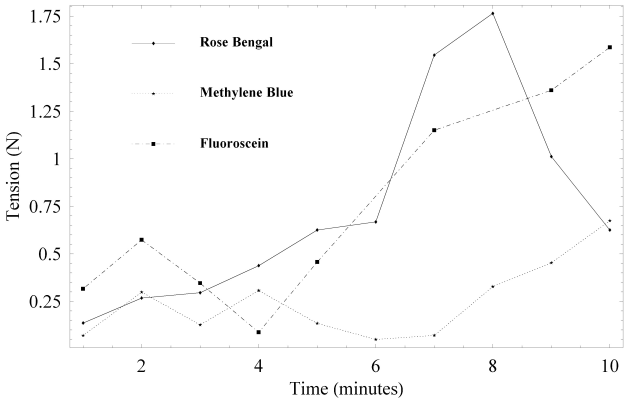

FIGURE 4. Maximum tension strength as a function of irradiation time.

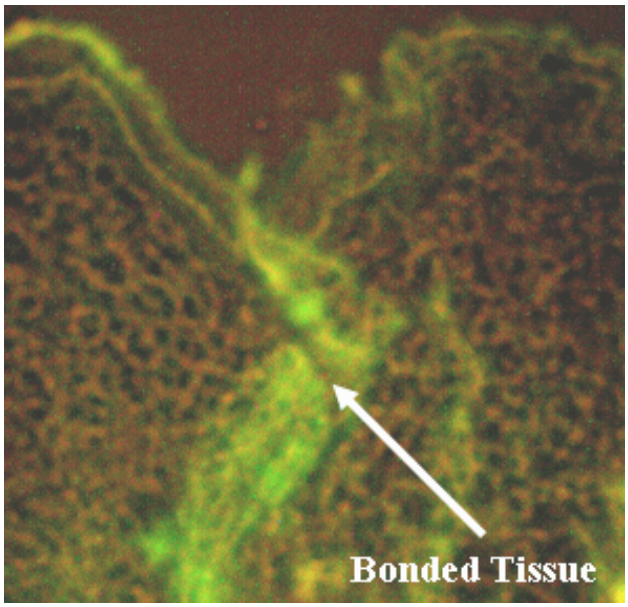

FIGURE 5. Epifluorescence micrograph of bonded skin tissue.

The skin was cut using a #15 scalpel into 1 cm × 2 cm pieces. The bonding procedure consisted in the application of the bonding agent to the walls of the skin pieces using a

cotton swab, the bonding agents used were a 1% solution of methylene blue in water, a 0.01M solution of rose Bengal in phosphate buffer solution (PBS) and a 0.01M solution of fluoroscein in PBS. The bonding agent was allowed to rest for 30 seconds and then irradiated with the light source at an irradiance of 0.1 W/cm^2.

To assess the bonding strength, the skin was tested with a tensiometer 10 minutes after irradiation. The maximum load at which the bonding failed was recorded.

Temperature measurements were performed on the irradiated skin using an infrared camera with a thermal sensitivity of 0.1°C (FlexCam-S, Infrared Solutions Inc, Plymouth MN), in order to assess the possible heat-related skin damage.

3. Results

To assess the strength of the bonded tissue, each sample was tested 10 minutes after repair with a calibrated tensiometer. Figure 4 shows the maximum tension before the bonding failed as a function of the irradiation time, these measurements show that non-laser photochemical bonding is as strong as previously published laser-irradiated experiments [6]. Based on the results shown in Fig. 4, it can be seen that there is an activation time where the bonding agent starts to be effective, and that this activation time is approximately 4 minutes for rose bengal and methylene blue, and 6 minutes for fluoroscein at an irradiance of 0.1 W/cm^2. If the bonded tissue is irradiated for a period of time greater than the activation time, then the maximum tension that the bonded tissue can stand increases linearly; in the case of rose bengal after 8 minutes of irradiation time a decrease in the maximum tension was observed, which may be due to thermal effects that affect this specific dye.

Figure 5 shows an epifluorescence micrograph of one of the skin samples bonded with the non-laser light source, the sample was cut in 4 μm slices using a crytome and then imaged with an epifluorescence microscope. From the micrograph it can be seen how there is effective cross-linking in the dermis, where most of the collagen fibers are located.

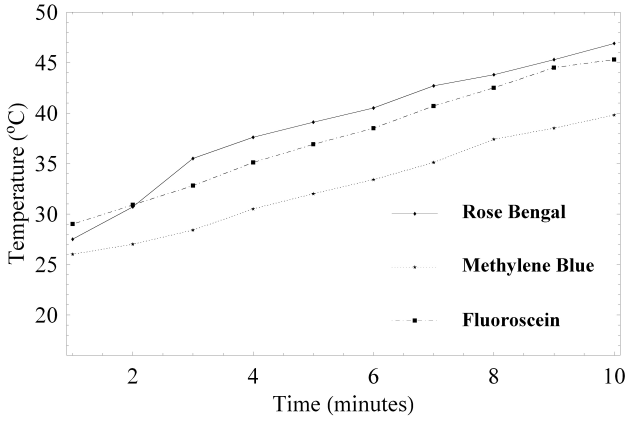

FIGURE 6. Measured temperature as a function of irradiation time.

Figure 6 shows the measured temperature as a function of the irradiation time, the temperatures measured are well below the critical temperature for cell death, which is about 60°C for short periods of heating [6]. These temperatures are expected to be lower *in vivo* due to thermal regulation in highly perfused skin.

4. Conclusions

A non-laser approach to photochemical tissue bonding has been tested using three different bonding agents (methylene blue, rose bengal and fluoroscein) on human skin. Tension measurements showed that a non-laser light source can produce as good photochemical skin adherences as can its laser-irradiated counterpart. Skin temperature measurements showed that temperature increase due to the light source does not induce thermal-related skin damage.

Acknowledgments

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