Original Article

Photoactivation of Autologous Materials with a New Reliable, Safe and Effective Set-Up

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Abstract

Background: The possibility of improving conditions and pathologies using biological materials prepared with the patient's own tissues has always been an attractive idea. There is a great disparity between the huge amount of preclinical data and the limited research conducted on photomodulation or photoactivation. This is because, for an effective and controlled management of light energy, several obstacles must be overcome.

Aim: The aim of this study is to evaluate the physical obstacles encountered by light in its path from the source to the biological tissue lodged in a receptacle specifically built for this purpose.

Methods: Total reflectance (specular + diffuse for an incidence angle of 8o) and total transmittance (regular + diffuse) of a rectangular area of 2 cm2 corresponding to a 5-cm long, 4-cm wide, 1-mm thick Terlux 2812HD plastic polymer sheet were evaluated.

Results: Showed that, with this set-up, over 90% of emitted light energy reaches the targeted tissue, with less than 10% loss in the process.

Conclusion: Data obtained in this study enable us to establish the suitability of this system as an effective tool to take advantage of the clinical benefit of photoactivation of biological materials.

Keywords

Autografting, cell transplantation, light, photoactivation, photomodulation

Abbreviations: LEDs, light-emitting diodes; CSIC, Consejo Superior de Investigaciones Científicas (Spanish National Research Council)

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Introduction

Regenerative Medicine is an emerging interdisciplinary field of research with clinical applications, focused on repairing, repleting or regenerating cells, tissues or organs in order to restore damaged function[1].
The possibility of healing or improving conditions and pathologies through regenerative medicine by using biological materials prepared with the patient's own tissues has always been a very attractive idea. That fantasy became a reality in 1958, when the first report on an autologous hematopoietic cell transplant attempt was published[2].
Only two decades later, the first reports regarding the healing of pathologies that had been previously considered incurable appeared[3,4].
At the beginning of the 60s, the first successful trials with stem cells on animals were published, continuing for the next 20 years.
The first attempts on humans failed[5,6] but, during the 80s, these treatments became established[7,8]; and, half a century after the first attempts, autologous transplants became a versatile medical resource, used for several purposes and with a high frequency.
In the last decade, autologous materials, such as plasma or serum with high concentrations of growth factors and anti-inflammatory cytokines, have been massively used[9]. The simple manipulation of a small amount of the patient´s blood allowed physicians to deliver good therapeutic effects through different administration routes, such as: ocular[10], intramuscular[11], epidural perineural[12], intra-articular[13], or transdermal[14], and for a myriad of medical specialties. The possibility of processing our own blood in order to obtain precious substances for a particular purpose opened the door for the development of new treatments, indications and techniques. But, eventually, the amount of improvements regarding the general use of these materials slowed down dramatically until the present day, when the game-changing concept of "conditioning" appears.
Autologous materials can be conditioned.
In this context, conditioning stands for the controlled exposure of the autologous material to a certain physical and/or chemical stimulus, relying on the fact that the exposure itself will determine changes in the material that will ultimately lead to an enhancement of its clinical capabilities and curative potential.
The field of action of conditioning of autologous materials, of biostimulation or biomodulation, and of biomaterial activation is extremely wide. One of the conditioning methods that has been more researched in recent years is photostimulation or photomodulation.
This term includes all procedures performed with different light technologies, such as: lasers, light-emitting diodes (LEDs) and other types of lamps and/or emitters.
The action that light exerts on biological structures is based on the first law of photobiology, according to which light absorption requires the presence of a photoreceptor that, when excited, may induce activity through signaling cascades[15].
To explain this interaction, several mechanisms have been proposed, although there are studies showing results that suggest the important roles played by oxidative processes in biostimulation: increases in cell proliferation and in levels of oxygen reactive species after stimulating leukocytes using a 660-nm light and a dose of 0.5-5 J/cm2[16], and an increase in cell proliferation after stimulation of osteoblasts with a 980-nm light and blocking of said proliferation in the presence of an antioxidant agent[17].
Regardless of the molecular mechanism involved, it is accepted that light modifies cell function, such as that of fibroblasts, and accelerates the repair of connective tissue[18]. A high cell proliferation (significantly higher than the control group) has also been reported after stimulation of cells with several energies between 1.96 J/cm2 and 7.84 J/cm2[19].
It is generally accepted that the energy density that seems to induce an effective biostimulation or biological conditioning effect is extremely variable, ranging from magnitudes as different as 0.09 J/cm2 and 90 J/cm2, although the most frequently used values are within the range of 1-5 J/cm2[20].
The concomitance of a large amount of preclinical data and a very limited number of high-level studies conducted on human beings in the field of biophotomodulation is concerning. Concerning, but not surprising because, for an effective and controlled management of light energy, a fair number of physical obstacles must be overcome. First, tissues must be arranged in such a way as to ensure they are properly exposed to the light emitted. Second, in order to set the foundation of an accurate dosage and a future therapeutic protocol, exposure of the whole tissue must be homogeneous.
Furthermore, receptacles must be built and standardized with the proper chemical composition and geometry, allowing to ensure the efficacy of the stimulus administered and patients' safety.
Lastly, the technology containing a light emitter able to provide energy to the receptacle in a proper and safe way must be built.
In order to overcome these physical obstacles and ensure a proper dosage to set the foundation of a photomodulation or photoactivation treatment, a receptacle was built (*Figure 1*).

Figure 1 *- Receptacle designed to photoactivate 10 ml of liquid autologous tissue. Specifications can be found in the main text.*

Specifically designed, it is mostly made of a medical-grade synthetic polymer called Terlux 2812HD (it contains other components in less degree, which have not been mentioned for industrial protection reasons). Besides its special chemical composition, its geometry has been conceived as to maximize the interface with the light source, allowing proper exposure of the whole tissue to the light.

Finally, the dimensions of the light source and the way the receptacle has been arranged inside have allowed to provide the receptacle itself with very thin walls (1 mm) and a camera that, with very few mm of depth, is able to lodge 10 ml of liquid biological material inside.

These characteristics enable us to stimulate a fairly appropriate volume of tissue for treatment, and at the same time minimize the turbulent flow of the material inside for proper homogenization of the dose.

The aim of this study has been to evaluate the capability of the emitted light (280-1500 nm) to go through the medical-grade synthetic polymers that constitute the receptacle.

Methods

Total reflectance (specular + diffuse for an incidence angle of 8o) and total transmittance (regular + diffuse) of a rectangular area of 2 cm2 corresponding to a 5-cm long, 4-cm wide, 1-mm thick Terlux 2812HD plastic polymer sheet were evaluated.

For this, a double-beam spectrophotometer (Perkin Elmer, Lambda 1050) was used, with a diffuse reflectance accessory provided with an integrating sphere painted on the inside with barium sulfate (measurement geometry: 0o:d, including the specular component of reflectance). A method of measurement by comparison with a diffuse reflectance pattern was used.

Spectral reflectance has been measured in the interval from 280.0 nm to 1,500.0 nm, with a 2.0-nm bandwidth in the ultraviolet and visible spectra, and with a variable bandwidth in the infrared spectrum.

The mean of uncertainty for measurements was 0.02 (SD 0.02). BK97 (register number) was used as the reference pattern, taking the zero value of the instrument and using a light ramp instead of the sample.

Three independent sweeps were performed.

All measurements were conducted at the Institute of Optics "Daza de Valdés" Spanish National Research Council (CSIC), Madrid, based on their PTRA10 (Diffuse reflectance calibration procedure) technical procedure and under controlled environmental conditions (22.6oC +/- 0.5oC).

Transmittance and reflectance were not expressed in any unit because they were the result of the quotient of two radiant fluxes: the incident and transmitted fluxes (transmittance), and the incident and reflected fluxes (reflectance).

Results

The transmittance curve (*Figure 2*) produced a mean value of 85.83% (SD 13.05). When analyzed, three areas can be easily distinguished. The first area of the curve included the interval from 280 nm to 490 nm and shows an abrupt increase in transmittance values of 6.35% to 88.45%. The mean of this area was 69.33% (SD 24.98). The second area of the curve included the interval from 490 nm to 1,100 nm and had a mean value of 90.80% (SD 0.81). A plateau can be observed, with virtually constant transmittance values that fluctuated between 88.62% and 91.80%. Lastly, the third area of the curve included the interval from 1,100 nm to 1,500 nm and showed a mean value of 87.32% (SD 1.19). A small decrease in transmittance can be observed, with somewhat higher fluctuations that can reach a value of 85.69%.

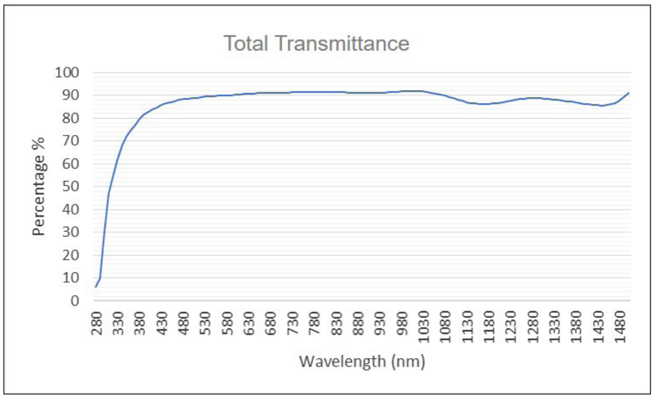

Figure 2 *- Curve generated using all measurements obtained from total transmittance.*

The analysis of the total reflectance curve shows a mean value of 8.87% (SD 0.62). Three areas can be distinguished in this curve as well (*Figure 3*). The first area of the curve included the interval from 280 nm to 380 nm. Here, measurement results increase until reaching the maximum peak of the whole sample: 10.68%. The mean value was 9.36% (SD 1.29). The second area of the curve included the interval from 380 nm to 670 nm, showing a gradual decrease in reflectance values until reaching 8.94%. The mean value is 9.46% (SD 0.50).

Lastly, the third area of the curve includes the interval from 670 nm to 1,500 nm, where fluctuations of values decreasing until reaching 8.19% can be observed.

The mean value was 8.60% (SD 0.23).

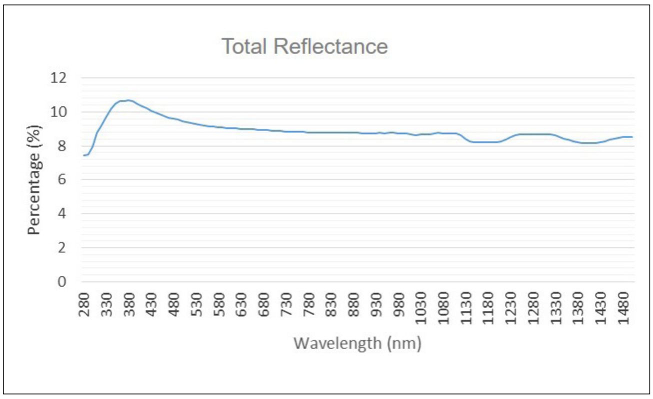

Figure 3 *- Curve generated using all measurements obtained from total reflectance.*

Discussion

Results show that over 90% of emitted light energy reaches the targeted tissue, with less than 10% being lost in the process. This is the same for all wavelengths between 450 nm and 1,450 nm, thus providing this treatment with huge versatility and potential. Taking into account that with the data obtained from this study, we can accurately measure the reduction of energy reaching the target, a simple calculation will allow us to adjust the light source emission to the required dose with precision. That is to say, this setup (the receptacle and the emitting source) ensures two fundamental facts: that light energy, both in quantity (dosimetry) and quality (wavelength/light), is reliable, controlled and measurable; and that we can accurately control the amount of energy absorbed by the tissue. Both facts will ultimately allow to establish effective therapeutic protocols for photomodulation or photoactivation.
However, it is worth noting that these results make no mention of the true clinical potential that photomodulation or photoactivation has or may have.

Conclusion

Data obtained in this study enable us to establish the suitability of this system as an effective tool to take advantage of the clinical benefit of photoactivation of biological materials. Future clinical studies must assess the clinical benefit of this treatment and transform this innovative, reliable tool in effective therapeutic protocols that are able to provide benefits for patients in endless clinical contexts. From here on, a huge range of possibilities opens up, where each specialist can suggest, with guarantees, the use of photomodulation or photoactivation in a safe and reliable way for different pathologies and with different goals.

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Author contributions

HP was involved in conceptualization, investigation, writing-original draft, project management.

Conflict of interest

The author declares no financial or commercial conflict of interest.

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