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GENERAL AND APPLIED PHYSICS

The Physics of Light and Sound in the Fight Against Skin Cancer

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Abstract
Multidisciplinary work is necessary for the development and improvement of techniques for the diagnosis and treatment of diseases, with physics playing a prominent role. For example, in-depth knowledge of the interaction of electromagnetic (ionizing and non-ionizing) and mechanical (sound and ultrasound) waves with biological tissues is important for the development of protocols for the management of skin cancer. In this paper, we review the developments carried out at the São Carlos Institute of Physics that resulted in new technologies and protocols for the diagnosis and treatment of non-melanoma skin lesions. Photodynamic therapy (PDT), a technique that combines light and a photosensitive molecule, was improved, and more than 2000 patients were directly treated in our clinical trials. Our research group pioneered the technique in Brazil, and we developed several fundamental studies involving cell culture and animal studies that demonstrated the effectiveness of this technique in treating cancer. Based on these results, multicenter clinical studies in Brazil have been implemented for the treatment of non-melanoma skin cancer. These studies generated commercial prodrugs and irradiation devices that are currently being considered for incorporation into the Brazilian public health system. In addition, nine countries in Latin America have implemented our technique. In this paper, we also show the physical fundamentals of PDT, the new developments that will allow us to overcome current challenges such as the use of ultrasound to treat cancer. Developments in the diagnosis of cancer will also be discussed.

Keywords Cancer · Treatment · Optics · Acoustics · Biophysics · Photodynamic · Sonodynamic

1 Skin Cancer

Despite recent efforts and scientific advances over the years, cancer remains one of the top three causes of death globally. This is aggravated by an increase in life expectancy, population growth, exposure to carcinogens, and lack of medical assistance [1, 2].

Currently, the leading techniques for cancer treatment are surgery, chemotherapy, and radiotherapy. Despite having good results, these techniques have significant disadvantages. For example, surgery often removes healthy tissue, resulting in cosmetic damage besides being a traumatic procedure. Despite focusing and delivering high doses of radiation into the target tissue, radiation used in radiotherapy passes through healthy tissues, resulting in irritation [3]. Furthermore, radiotherapy and chemotherapy cause a decrease in the patient's immunity, making him susceptible to contracting other diseases [4]. Therefore, one of the greatest challenges of the twenty-first

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century is to develop safe and efficient techniques to treat cancer with minimal side effects.

Skin cancer is the most commonly occurring type of cancer and can be categorized as either melanoma skin cancer or non-melanoma skin cancer (NMSC). Melanomas are the most aggressive skin cancer, with the greatest potential for metastasis, although they have the lowest incidence. Cutaneous melanoma is usually a visible and pigmented lesion that obeys the ABCD rule: asymmetry, border irregularity, color variegation or a dark black color, and a diameter greater than 0.6 cm. Early diagnosis increases the patient's chances of successful treatment [5]. A melanoma develops due to genetic abnormalities within the melanocyte, which promotes cell proliferation and prevents the normal pathways of apoptosis. Altered melanocytes are predisposed to cumulative DNA damage and genetic mutations, allowing the development of malignant phenotypes, such as angiogenesis, immune response evasion, tumor invasion, and metastasis [6]. In contrast, NMSC corresponds to approximately 30% of all malignant tumors registered in Brazil, with an estimated 177,000 new cases in 2020, according to the Brazilian National Cancer Institute (INCA) [7]. The World Health Organization's 2020 database ranked Brazil as the country with the seventh highest number of NMSC cases worldwide [8]. However, unlike melanoma, NMSC has a low mortality rate. Basal cell carcinoma (BCC), a type of NMSC, has the highest incidence and accounts for 67% of cases. BCC is a cutaneous malignant proliferation that is derived from the basal cell layer and outer root sheath of hair follicles, the so-called pluripotent epithelial cells [9, 10]. Squamous cell carcinoma (SCC) is also a type of NMSC and has the second highest incidence rate which corresponds to 33% of all cases. Conventionally, SCC is characterized by atypical cells in the dermis and the presence of enlarged and pleomorphic nuclei, and abnormal, accelerated growth of squamous cells. The SCC classification is based on the degree of tumor differentiation, where grades I, II, III, and IV include tumors composed of $< 25\%$, $< 50\%$, $< 75\%$, and $> 75\%$ undifferentiated cells, respectively [9, 10].

Surgical removal is considered the gold standard in the treatment of NMSC lesions. However, the use of surgery as a treatment option is difficult in developing countries due to the high incidence of lesions and the need for infrastructure and specialist doctors. Surgery has a success rate of 96% in the treatment of BCC lesions [11]. However, although surgery is more effective, it has poorer cosmetic results than topical treatments, especially since most lesions are located on the head and neck [12]. In addition, surgery commonly has serious adverse effects such as local infections, causing the need for secondary treatment [11, 13].

2 Interactions with Biological Tissue

2.1 Electromagnetic Waves

Electromagnetic waves are formed by oscillations of electric and magnetic fields, which can either propagate in a vacuum or another medium, such as biological tissues. This radiation can be quantized, with the smallest unit of energy being the photon. To characterize these waves, we use quantity, frequency, and wavelength; thus, we have the possibility of waves with different frequencies, consequently resulting in a specific energy (photon). The electromagnetic spectrum is made up of all these frequencies, ranging from low-frequency (radio waves), through intermediate-frequency (microwaves and infrared), to high-frequency (visible light, ultraviolet, and X-rays). The visible light can be detected by the human eye. Some ultraviolet radiation and X-rays are referred to as ionizing and are capable of ionizing atoms and molecules, while the remainder of the spectrum is non-ionizing [14].

Each region of this spectrum has its generation, detection, and interaction characteristics with matter. Specifically, regarding biological tissues, each region of the spectrum has a depth of interaction as shown in Fig. 1. This depth is related to the phenomenon of this interaction, mainly, absorption and scattering.

After being generated by a source, electromagnetic radiation propagates until it reaches biological tissue. Because of the change from the propagation medium to the biological medium, specular reflection occurs at this interface; namely, the entry and exit angles of the electromagnetic radiation relative to the interface normal are equal. Part of this radiation is refracted when traveling through different layers of

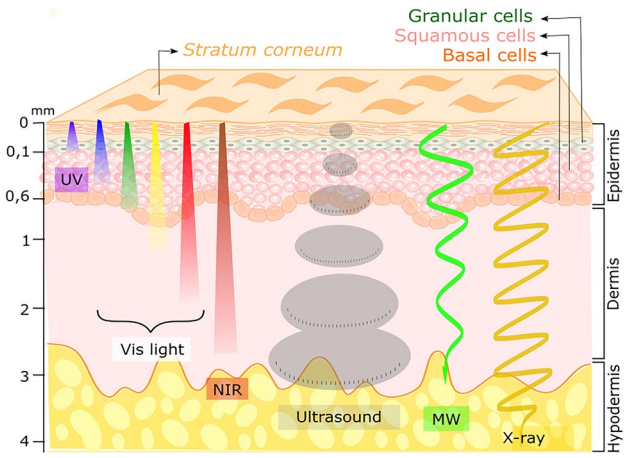

Fig. 1 Penetration depth of different sources through the skin tissue. Light with a longer wavelength propagates through the tissue more deeply. *UV* ultraviolet, *Vis* visible, *NIR* near-infrared, *MW* microwave

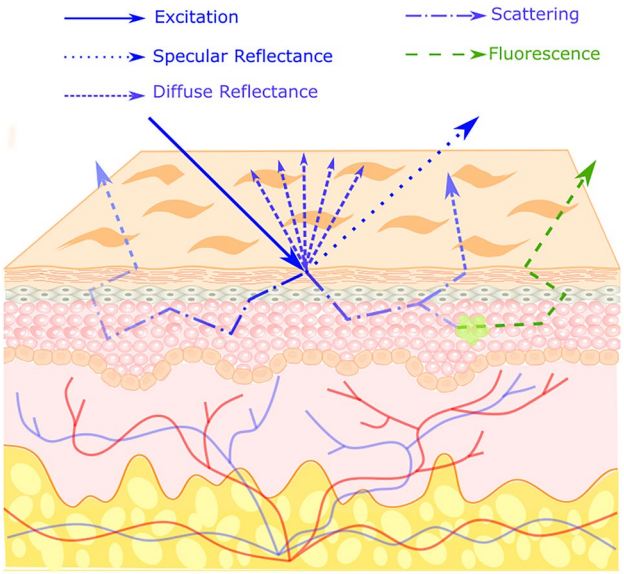

Fig. 2 Light-tissue interactions

the biological medium as well as scattered or absorbed when it propagates through the tissue. Each wavelength of electromagnetic radiation exhibits a different interaction with the tissue (scattering and absorption). Eventually, scattered light within the tissue emerges in the form of diffuse reflectance. Within the tissue, this radiation interacts and decreases in intensity. In some cases, the molecule can emit fluorescence after absorption, which also emerges from the tissue [14]. These primary phenomena of radiation interaction with tissues are illustrated in Fig. 2.

2.2 Mechanical Waves - Ultrasound

Unlike light, an acoustic wave is not an electromagnetic wave but a mechanical wave; therefore, it always requires a physical medium to propagate [15]. Ultrasound is an acoustic/mechanical wave that, compared to light, penetrates deeper into biological tissue, to a depth of a few centimeters [16].

In medicine, therapeutic ultrasound is generated by a device called an ultrasonic transducer, through a reverse piezoelectric effect. This effect occurs when an alternating current is applied to a piezoelectric crystal placed in the transducer, causing crystal vibrations that result in the transmission of mechanical/acoustic energy to the therapeutic target [15].

When the ultrasound beam strikes an acoustic interface, that is, a surface with two materials of different densities (e.g., different tissue layers), the course of the ultrasound is influenced by the degree of change in density between the two tissues. Therefore, reflection and refraction can occur, similar to those that occur with electromagnetic radiation (Fig. 2).

Ultrasound waves can also be scattered because of their interaction with cells and tissues that are smaller than the wavelength of ultrasound. There is also the possibility of absorption due to the intrinsic internal friction of the propagation medium. The energy of ultrasound waves decreases each time they undergo these physical phenomena during their propagation through different tissue interfaces, which is known as attenuation [17]. The ultrasound penetration depth within biological tissue is also attenuated by the sensitivity of the ultrasound, which is inverse to the wave frequency [15]. Lower frequencies penetrate deeper into the tissues. In medical diagnosis, a frequency of approximately 2.5–15 MHz and a lower amplitude are used. In therapeutic applications, it is more common to use high amplitudes and frequencies of approximately 0.75–3.3 MHz [15].

Another well-known phenomenon induced by ultrasound within a fluid is acoustic cavitation. It involves the formation, growth, and collapse of microbubbles (small gas microspheres of 1–8 μm in diameter) due the pressure pulses created by ultrasound waves. [18, 19] Acoustic cavitation can be classified as non-inertial or stable cavitation and inertial or transient cavitation, according the behavior of the microbubbles (Fig. 3). Stable cavitation refers to the expand and contract of bubbles with stable oscillation around the same resting radius with long lifetime. In nearby environments, stable cavitation is able to create shear stress and microstreaming. On the other hand, inertial cavitation refers to the very rapid growth of microbubbles until reach a critical upper size where the liquid rushes in and the cavity implodes in its immediate vicinity. The bubble implosion gave rise to several mechanical forces such us microstreaming, microjetting, and shock waves, as well as locally short-lived high levels of temperature (4000–25,000 K) and pressure (above 800 atm) called hot spots. The hot spots may trigger sonochemical effects such us sonoluminescence phenomenon, this means the emission of rapid peaks of light, and thermolysis of the water vapor inside the bubble [17, 20, 21]. Due to such sonomechanical and sonochemical effects, ultrasound is being conveniently used in the therapeutic area, with

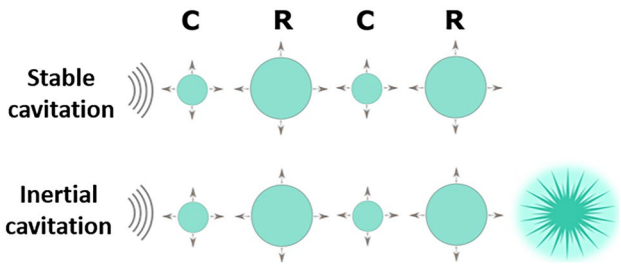

Fig. 3 Acoustic cavitation can be classified as stable cavitation and inertial cavitation. *C* compression phase, *R* rarefaction phase

sonodynamic therapy (SDT) being one of its most recent therapeutic approaches of growing interest and research.

3 Dynamic Therapies

Dynamic therapy refers to anticancer therapeutic approaches based on reactive oxygen species (ROS) generation that lead to tumor cell necrosis or apoptosis. ROS are a group of chemically reactive oxygen-containing radicals (superoxide anion O_2^-, hydroxyl radical •OH) and nonradical derivatives (hydrogen peroxide H_2O_2, singlet oxygen $^1O_2$) that are capable of inducing cell death when its intracellular accumulation is high (Fig. 4). The emerging therapies included in this group are photodynamic therapy (PDT), radiodynamic therapy (RDT), microwave dynamic therapy (MDT), and thermodynamic therapy (TDT); these techniques apply a electromagnetic energy source; SDT that apply a mechanical energy source; electrodynamic therapy (EDT) that apply an electric energy source; and chemodynamic therapy (CDT) based on the chemical reactions triggered by the inclusion of various molecules. All of these techniques operate on the same principle; an energy source interacts with a molecule sensitive to such a wave leading a series of reactions resulting in ROS production [22].

A specific type of molecule is needed for each technique. Light-activated or light-sensitive molecules are called photosensitizers (PS), whereas ultrasound-activated molecules are called sonosensitizers (SS) [23]. Similarly, the molecules used in the other dynamic treatments will take a name related to the exciting source applied in such a treatment (e.g., microwave sensitizers, radiosensitizers, thermosensitizers, electrosensitizers). All of these therapies are summarized in Fig. 4.

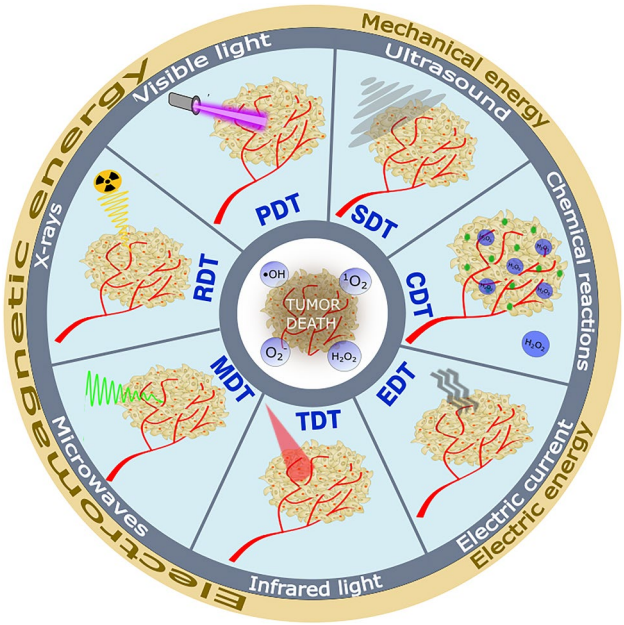

Fig. 4 Schematic illustration of X-dynamic therapies. PDT is based on the interaction of light and PS (e.g., porphyrins, phthalocyanines, indocyanine dyes, curcumin); sonodynamic therapy (SDT) is based on the combination of ultrasound waves and SS (e.g., porphyrins, methylene blue, curcumin, TiO_2, $MnWO_x$ NPs) [23–27]; radiodynamic therapy (RDT) combines PDT and radiotherapy through the use of X-rays or gamma rays to activate photosensitizers [28]; microwave dynamic therapy (MDT) involves the combination of microwaves and microwaves-responsive agents (e.g., copper-cysteamine (Cu-Cy) nanoparticles, TiO_2 nanoparticles, Mn-doped zirconium metal–organic framework nanocubes, gold nanoparticles) [29]; thermodynamic therapy (TDT) is based on the reactions triggered by the conjunction of hyperthermia and thermosensitizers (e.g., anthracene endoperoxide derivative, azo initiators) [30]; electrodynamic therapy (EDT) applies electric current energy and sensitizers(e.g., platinum nanoparticles [31]) as components; and chemodynamic therapy (CDT) based on chemical reactions triggered by the inclusion of various molecules [22]. H_2O_2 hydrogen peroxide, $MnWO_x$ NPs ultrasmall oxygen-deficient bimetallic oxide $MnWO_x$ nanoparticles, TiO_2 titanium dioxide

3.1 Photodynamic Therapy

PDT is a therapeutic technique mainly based on the excitation of a PS with light of an appropriate wavelength, resulting in the production of ROS in the cell tissue, which is highly cytotoxic and causes cell death [32, 33]. In the biological tissue, one first important thing is the light propagation. The absorption and the scattering of each tissue and lesion need to be taken into account, to deliver the right amount of energy into the target tissue. Details of light propagation in tissue can be found in the following reviews [34, 35]. Once the light is in the tissue, it will also be absorbed by the PS, which needs to be in the target tissue with the proper concentration. Many molecules or nanostructures can be used as PS, and its function is to transfer energy from light to some intrinsic molecules into the tissue that will form ROS [36]. To produce irreversible damage in a cell, it is necessary to procure a concentration of ROS above a threshold. The amount of ROS produced depends on the quantum yields of the PS and the light fluence delivery into the PS localization. All the processes of the light interacting with the PS can be described by the Jablonski diagram and for some rate equations that can be found elsewhere [37–39]. The reactive species generated by dynamic therapies, in general, can cause the target cell death by different mechanisms. The main three via are apoptotic, necrotic, and autophagy. The preferential via is mostly related to the subcellular localization of the PS in different organelles. Besides that, other factors can favor one of the death mechanisms such as PS concentration, light fluence, and drug-light interval [40]. Several basic studies have been developed by our group to better understand the interaction of light with PSs [41], the correlation between PS fluorescence and PDT outcome [42], light dose threshold [43], and light distribution in tissues, among others [44–46].

These fundamental studies paved the way to use PDT as an option for topical treatment of NMSC lesions. Compared to surgery, PDT has a lower cost, is easier to apply, requires a simple infrastructure, and promotes an excellent cosmetic response. Medical guidelines already recommend topical PDT for small BCC, Bowen's disease (in situ squamous cell carcinoma), and premalignant lesions such as actinic keratosis [47, 48].

We have been conducting studies using PDT for the treatment of lesions since the early 2000s, particularly at the Amaral Carvalho Hospital, which specializes in oncology. During initial studies, systemic PDT was used for SCC lesions in patients who were not eligible for surgery. Over 400 patients were treated, and approximately 80% of the lesions were located on the head, neck, or skin, but experience was also gained in the treatment of esophageal, bladder, gynecologic, and cutaneous recurrence of breast cancer, among others [49–51]. The use of PDT in treating Bowen's disease showed faster healing and less complications compared to surgery [52]. Other additional studies using systemic PS showed promising results in the treatment of NMSCs [53].

To incorporate the use of the technique in the Brazilian Public Health System, a national multicenter study called PDT Brazil was developed. In this study, approximately 1600 small NMSC lesions were treated with topical PDT. Approximately 70 centers in Brazil and other Latin American countries were trained to perform this procedure. Governmental financial support and collaboration with two university spin-off companies were responsible for producing irradiation devices and prodrugs. PDT Pharma was the company responsible for producing prodrugs for PDT Brazil. They produce protoporphyrin IX (PpIX) precursors such as 5-aminolevulinic acid (ALA) and methyl aminolevulinate (MAL) as either a powder or cream. They also provide other PSs, such as methylene blue and curcumin, which have been used for other PDT applications [54, 55]. The irradiation device created for PDT Brazil by the MMOptics Company has the main advantage of having both probes on the same platform: one for treatment and the other for monitoring. For monitoring treatment, the company also developed a wireless version of the probe called EVINCE (MMOptics, São Carlos, Brazil) [56–58]. The protocol applied at PDT Brazil is indicative of BCC, Bowen's disease, and actinic keratosis, with a diameter smaller than 2 cm (superficial lesions) and up to 2-mm thick (nodular lesions). The lesions were debulked, after which cream containing 20% methyl aminolevulinate (MAL—PDT Pharma, Cravinhos, Brazil) was applied for 3 h, thus providing the necessary time for the cells to convert the pro-drugs (MAL) into PpIX, which is an endogenous PS. They were then irradiated using a 630-nm LINCE system (MMOptics, São Carlos, Brazil) set at 125 mW/cm^2 for 20 min, totaling 150 J/cm^2 fluences in 3 cm^2 of irradiation area. A second session was performed after 1 week. The irradiation parameters were the same for both sessions. We used MAL cream exposed to light for 3 h, for incubation. A biopsy was performed 30 days after treatment to evaluate the possibility of a residual lesion, and 6 months after PDT, a clinical evaluation was performed to observe lesion recurrence. The results showed the complete removal of up to 86% of the treated lesions [54, 59]. In the PDT Brazil project, our research team was mainly responsible for enabling medical partners and supplying irradiation devices and prodrugs. Initially, we had 27 centers in the country using this protocol, and hundreds of BCC lesions were treated [60]. We also increased our scientific and clinical network by collaborating with other countries in Latin America, where we also provided them with irradiation devices, drugs, and training [55, 61]. Buzzá et al. specifically show the overall results of the PDT Brazil initiative, which after 6 years, still had forty-two active centers operating [62].

There have been many studies related to protocol optimization, one of which is referred to as "single-visit." In this study, several irradiation protocols were explored to achieve the highest cure rate by performing two PDT sessions on the same day. This is important to obtain greater patient acceptance and to make the treatment more comfortable. The first part of the protocol was the same as that used in PDT Brazil. However, for the single-visit protocol, the cream was re-applied shortly after the first irradiation and incubated for 1.5 h, followed by the second irradiation. Both types of irradiations had the same parameters. A biopsy and clinical evaluation were performed following the standard protocol. The single-visit protocol achieved a 95% cure rate, which was determined by examining the results of the biopsies collected 30 days after treatment. The patients treated were monitored for 5 years, and the cure rate remained similar to that of the standard protocol. This was of importance because we achieved similar efficiency while promoting a more comfortable and practical treatment [54, 59, 63, 64].

Skin cancer is far from being a problem only in Brazil or Latin America. Even countries like the USA and all European countries live with problems related to non-melanoma skin cancer [65]. Often, the problem is not just related to the cure rate, but the handling. Even rich countries like the USA have serious problems dealing with skin cancer. People often need to move from place to place, and this involves professionals and high costs. Even institutions consolidated in cancer, as is the case of The University of Texas MD Anderson Cancer Center in Houston, TX, believe that treating skin cancer is still expensive and the high numbers of lesions complicate the situation. In this sense, the creation of a platform that improves the procedure and allows facilitating the cure of skin cancer and even precancerous lesions such as keratosis, can have an impact across borders. In fact,

the developed system, which contains on the same platform the ability to diagnose, treat, and monitor the treatment to verify parameter adjustments during treatment, is an important component in this regard. The developed LINCE system (MMOptics, São Carlos, Brazil) platform allows this procedure to be carried out with low cost, safety, and ease of use. In this way, the project ended up having a worldwide impact. In addition to the technique having already been approved in Mexico to be performed, there are also several locations in the USA and Europe (France and Scotland) using the system and treatment platform [66]. The International Photodynamic Association (IPA) considers the Brazilian project as an example to be followed for the implementation of new technologies. In 2019, the IPA awarded our group with the Humanitarian Award due to our efforts in the development of technologies and protocols in photodynamic therapy and clinical implementation, especially in low-resource settings [67]. UNESCO also has an equivalent position, through the election of one of the program participants as a personality focused on applications of light in the areas of health care [68]. In addition, in November 2021, the "Month of Healthy Skin," our team's researchers are featured among the world's leading experts in the area of "photosensitizing agents" according to "Expertscape"—an agency that ranks scientists and institutions around the world through their work on more than 29,000 biomedical topics [69]. Certainly, the Global impact exists and is growing, and being adopted within the social medicine of Brazil, through the approval of the publicly funded unified health system in Brazil, it should have an enormous repercussion, with an even greater global impact.

Topical PDT is an effective, safe, and minimally invasive procedure, with excellent cosmetic results for the treatment of NMSC lesions. However, light propagation depends on its interaction with tissue structures, limiting its penetration depth to a few millimeters (see Fig. 1), so, tumors with greater skin infiltration (deeply seated tumor) have a limited cure rate. Another type of skin lesion not indicated to be treated by topical PDT is the pigmented lesions since they absorb even more incident light, making it difficult to establish effective protocols for the treatment of these types of lesions, especially melanoma that requires surgical removal, which is invasive, is aggressive, and has a poorer aesthetic result [70].

3.2 Sonodynamic Therapy

An alternative method to overcome these challenges is SDT, a minimally invasive anticancer modality that consists in the combined use of low-frequency ultrasound waves (0.75–3.3 MHz) and a SS, an acoustically susceptible molecule that is activated by ultrasound [23, 71].Unlike light, ultrasound has a low attenuation through biological tissues, so it propagates easily through them allowing this therapy to reach deeper layers (Fig. 1) [38, 72].

Similar to the clinical PDT protocol, SDT is based on systemic or topical administration of SS in the tumor tissue, followed by ultrasound irradiation. Finally, the sonodynamic reaction resulting from the interaction of the ultrasound and the SS triggers sonomechanical and sonochemical reactions that lead to the eradication of the target cells (Fig. 5) [16, 73].

Therapeutic US is widely used in physiotherapy and induces a series of physiological effects, such as increased blood circulation and oxygenation, among others [74, 75]. Direct necrosis by US is the technique known as HIFU (high-intensity focused ultrasound); this procedure is primarily based on the thermal effects of US [76]. In the case of SDT, the isolated application of US or SS will have no effect on the target tissue, only the correct combination of US and SS will cause the target tissue to die.

Although the mechanisms underlying the SDT-mediate cytotoxic effects are not fully understood, three potential mechanisms based on the phenomenon of acoustic cavitation could explain such effects. The first mechanism involves the interaction between several mechanical forces (e.g., microstreaming, microjetting, and shock waves) and the cell membrane destabilized by SS absorption, which leads to cell membrane disruption or even cell death [19, 77]. The second proposed mechanism is based on the sonoluminescence phenomenon. The emission of the sonoluminescent light photoactivates the SS following the same action mechanism as

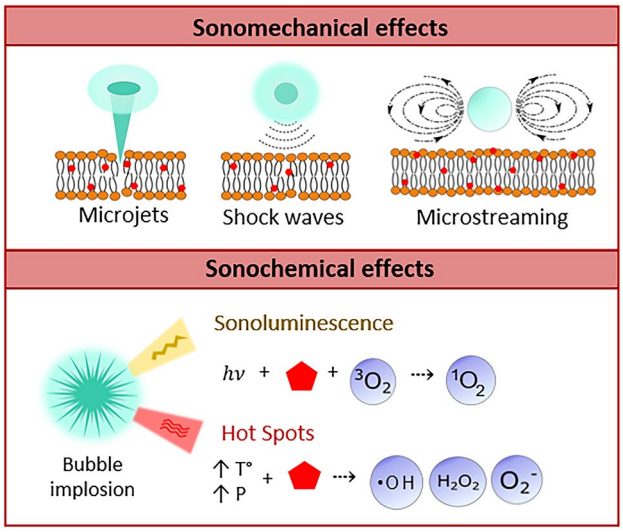

Fig. 5 Potential mechanisms of the SDT-mediate cytotoxic effects: The interaction of microjets, shock waves, and microstreaming and the cell membrane destabilized by SS absorption (sonomechanical effects); the interaction of the sonoluminescent light with the SS (sonochemical effects); and the interaction of the hot spots with the SS (sonochemical effects)

PDT [78]. The third mechanism involves the action of the high-temperature and high-pressure points generated during inertial cavitation, resulting in SS pyrolysis and the formation of highly cytotoxic and short-lived ROS, predominantly •OH, O_2^-, and H_2O_2 (Fig. 5) [16, 17, 73].

There are several studies around the world applying the SDT approach through the use of various sonosensitizers (e.g., 5-ALA, methylene blue, Rose bengal, and metal-based sensitizers) on different tumor models such as murine melanoma cells and xenografts. They have evaluated the SDT induced action mode by the study of the influence of this technique on the immune system, the cell death mode, the production of intracellular ROS, cell viability and tumor growth, getting promising results [79–84].

Furthermore, recent research by some groups in the world have shown a synergistic effect in the application of PDT combined with SDT in the treatment of deep-seated cancer (e.g., deep bowel and ovary) as well as pigmented tumors (e.g., melanoma tumors) [85–91]. This combination is called sono-photodynamic therapy (SPDT) and is a promising anticancer technique. The nature of the ultrasonic waves and the higher levels of ROS generated by SPDT than either PDT or SDT alone, are promising facts that make this technique one of growing interest in the scientific community.

In 2018, our research group started a new research line focused on the study of the use of therapeutic ultrasound in the treatment of cancer. In order to improve the understanding of the mechanisms behind SDT, our first steps involved a series of in vitro studies using PpIX as sensitizer; this molecule was used due to it has been reported as a good PS and SS. The experiments consisted of monitoring the absorption spectra of PpIX solutions during the exposure to ultrasound, varying the ultrasound intensity (1, 1.5, and 2 W/cm^2) and the initial PpIX concentration (1, 3, 5, 10, and 20 μM), in order to calculate the ultrasound-mediated PpIX decay rate for each case. We observed the formation of absorption bands at 280 nm after ultrasound irradiation of PpIX solutions, fact that did not occur during light irradiation, so it was called sonoproducts. It was found that the rate of sonoproducts production was independent of the concentration of the PpIX solutions, but directly dependent of the ultrasound intensity. Analyzing the behavior of the maximum absorption band of PpIX (i.e., Soret band at 407 nm) over the sonication time, we observe that the sonobleaching rate increased when it was used a lower initial PpIX concentration and a high ultrasound intensity. After analyzing the PpIX absorption spectra for each case, we deduced that, under the ultrasound parameters used, the sonoluminescence phenomenon may not be a predominant mechanism for the PpIX sonobleaching because of no photoproduct formation was observed at 635 nm in the absorption spectra [92]. So, It was assumed that the PpIX sonobleaching was mainly due to sonomechanical forces and the formation of ROS through pyrolysis of the sensitizer [23].

On the other hand, we compared the decay rate of 5 μM PpIX solutions exposed to light, ultrasound and both sources, in order to study the effects of the combined irradiation. It was found that the PpIX decay rate induced by the combined irradiation was approximately the sum of the decay rate induced by the light and ultrasound, showing to have an additive effect on PpIX degradation [23]. We also performed in vivo experiments to study the necrosis area produced in rat liver due to photodynamic, sonodynamic, and sono-photodynamic action using 5-ALA. These experiments showed that the sono-photodynamic activity triggered within the rat liver during the simultaneous light and ultrasound irradiation, induced cytotoxic effects at a greater depth of rat liver tissue, and a higher percentage of necrotic area in the longitudinal section of the liver than that induced by applying photodynamic activity [23].

Currently, our group presents several ongoing projects that aim to continue studying the ultrasound behavior and its effects in a biological environment, mathematically describe the cavitation phenomenon in a biological tissue based on ultrasound parameters and characteristics of the medium, determine the reactive oxygen species generated by different SS during its interaction with therapeutic ultrasound, and apply the SDT to overcome current challenges in the treatment of pigmented skin lesions.

4 New PS Delivery Ways

Other limitations of topical therapies include drug permeation into the skin, which can compromise successful treatment, especially for thicker lesions. Several studies have explored chemical or physical strategies to overcome this issue [93]. In terms of physical methods, there are common techniques for removing the stratum corneum and creating holes to improve drug permeation.

Tape stripping, for example, involves applying tape onto skin lesions and then pulling it off several times. It is a simple and safe procedure that is minimally invasive and inexpensive to remove the stratum corneum. A previous study compared PpIX homogeneity in an in vivo pig skin model. Fluorescence measurements showed that skin previously treated with tape stripping promoted more homogeneity in PpIX formation [94].

Debulking, or deep curettage, is another simple surgical procedure that is more often applied in thicker lesions, removing as much as possible of the superficial lesion from the total volume. Although debulking is more invasive than curettage, local anesthesia is not required. It has also been shown to be effective when applied either immediately or a few weeks before PDT, improving clinical response [95]. A clinical study performed by our research group showed that performing debulking before PDT can increase effectiveness

from 58 to 83% [53]. And consecutive studies have demonstrated protocols that have achieved up to 95.4% effectiveness [63].

The use of ablative fractional lasers to generate microchannels is another option for increasing the intensity and homogeneity of PpIX fluorescence in deeper skin regions [96]. Studies using a CO_2 laser associated with PDT for lesion pre-treatment showed an increase of 20% in the clearance rate [97–99]. Several types of microneedles have been used to enhance skin drug permeation. They are minimally invasive systems with simple application, are well accepted by patients, and do not cause pain or any bleeding [100–102]. With regard to material, microneedles can be classified as solid (metallic) or polymeric (hydrogel or dissolving). The use of metallic microneedles coupled with rollers for enhancing the penetration of topical cosmeceuticals has been widely described [103]. In PDT applications, some studies in pig models showed higher and deeper PpIX formation by using microneedle rollers compared with only topical application [104, 105].

Our research builds on these previous studies involving intradermal drug delivery using a dermograph. The device has nine metallic needles and is similar to a tattoo machine commonly used for aesthetic procedures, such as micropigmentation or cosmetics delivery. A pilot clinical trial was conducted to treat BCC lesions associated with dermograph therapy to PDT, and all six lesions remained healthy after 28 months [106]. Concerning the polymeric model of microneedles (MNs), a recent study by Requena et al. explored dissolving MNs containing ALA. This microneedle dissolves after insertion and allows deeper delivery of the drug. Preclinical results showed that PpIX fluorescence intensity was five times higher at a depth of 0.5 mm on average compared with the cream [107]. We are currently working to develop this technology in Brazil and expand the microneedle model to a clinical trial.

Another parameter that can help with drug distribution into the lesion is temperature; increased temperature during the incubation period can improve PDT effectiveness by increasing ALA penetration, increasing the amount of skin oxygen due to vasodilation, increasing the conversion rate of ALA to PpIX, and increasing ALA uptake in the cells. Stringasci et al. demonstrated that the most efficient way to increase PpIX production and penetration by temperature is to increase the local temperature before applying the cream [108].

PDT efficacy is also related to the presence of oxygen. In this context, vasodilation can be an important tool to increase oxygen supply and optimize treatment. A study was conducted by our research group to evaluate the association of menthol, methyl nicotinate, and ginger with ALA and MAL. These preclinical results show a decrease in incubation time and an increase in PpIX production. The best result was observed with the incorporation of methyl nicotinate with MAL, achieving 50% higher PpIX production compared to the cream containing only MAL [109].

There are other methods not explored by our research group that can be used to optimize drug delivery. Iontophoresis, for example, is a physical approach that improves transdermal drug delivery by facilitating the transport of ionic species across the cell membrane. It has been evaluated in preclinical and clinical studies demonstrating PDT improvement by reducing the drug light interval. However, more studies comparing PDT efficacy are necessary and there is a concern related to ensuring drug accumulation in the tumor without promoting systemic distribution causing undesirable side effects [110]. In this context, sonophoresis has been explored as well, showing higher ALA uptake in preclinical studies. The main difference is that sonophoresis facilitates drug transport under the influence of an ultrasonic perturbation (low frequencies) [111].

5 Photodiagnosis

In addition to efforts to treat non-melanoma skin cancer, several optical techniques have been developed to distinguish between clinically similar lesions. When light is absorbed by molecules, the electrons move from the ground state to an excited state. When an electron decays to the ground state, it can emit energy in the form of light. This process is called luminescence and is further classified as phosphorescence (with long lifetimes in the excited state) or fluorescence (with rapid relaxation to the ground state). Fluorescence can be analyzed in two ways: with steady-state and time-dependent techniques [112, 113].

We have conducted several studies using this technique to aid in the diagnosis of skin lesions. Wide-field fluorescence imaging is a steady-state technique that has shown promise when identifying lesion edges, particularly those of large areas. In addition, biological markers with absorption and emission at specific wavelengths, such as PS, which are selective for tumor cells, can be used to better visualize the lesion area [114, 115].

Spectroscopy is another steady-state fluorescence technique. Using spectroscopy, it is possible to obtain information on the entire visible spectrum of tissue emission in addition to greater signal depth, although this result is limited to a small point in the tissue. A study using spectroscopy showed that PpIX accumulation increased throughout the entire 3-h MAL incubation period and that PpIX production does not differ by lesion type, location, patient sex, or skin type [116]. Using fluorescence spectroscopy, it was also possible to demonstrate that photoaged skin has a different fluorescence from that of aged skin (higher and more heterogeneous), as it has some structural changes such as

epidermal hyperplasia and reduction of collagen fibers [117, 118]. Although many molecules emit fluorescence at the same wavelength, their respective decay times can differ, and time-dependent fluorescence can be used in these studies. However, most current fluorescence lifetime systems are not robust or portable, making clinical application unfeasible. A fluorescence lifetime spectroscopy system was assembled in a suitcase, with everything robustly encased, maintaining mechanical, electrical, and optical stability during transportation. The characterization and validation of the instrument were tested in a clinical study, where the system was shown to be reliable, and the results showed its potential use as an auxiliary tool for skin lesion diagnostics [119, 120]. A multispectral fluorescence lifetime imaging (FLIm) dermoscopy system was also developed and used to image nodular BCC (nBCC) lesions. Endogenous issues were imaged using three simultaneous emission bands: 390 ± 20 nm, 452 ± 22 nm, and > 496 nm to preferentially target three endogenous fluorophores: collagen, reduced nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FAD). Statistical classifiers were applied to differentiate BCC from healthy tissue, and the results showed the potential of the technology to be implemented as an image-guided tool to improve the border delimitation of nBCC during surgical resection [121].

Another imaging tool that has aroused great clinical interest is thermography, as nowadays its detectors present better resolution quality and greater sensitivity. With regard to tumor diagnosis, lesions have different temperatures according to their vascularization and metabolic activity. One study evaluated the use of thermography to differentiate between clinically similar lesions. An image processing routine using a support vector machine classifier showed the ability to differentiate SCC lesions from actinic keratoses in 80% of cases. In addition, thermography has shown promise in identifying secondary lesions of subcutaneous melanomas [122, 123]. A study was also carried out demonstrating the use of thermography as a tool for monitoring lesions treated with PDT; the images were processed and a model was created to calculate the amount of light energy that was converted in each process involved. Despite the approximations, it was shown that most of the energy deposited was converted into photodynamic action (53.8%), followed by the portion released by blood perfusion (37.2%), and no relevant thermal component was observed to generate tissue damage [124].

Image processing can be used to discriminate lesions using conventional white-light images. One study used images of 104 pigmented lesions obtained using standard digital cameras without lighting or scale control. The images were processed in terms of shape, asymmetry, and curvature, and the metrics obtained were used for identification. The results showed a sensitivity of 85% and specificity of 70% in detecting melanoma [125]. Another study used an optical system composed of four types of LEDs as a light source, white and RGB with emission in the red (maximum at 663 nm), green (maximum at 535 nm), and blue (maximum at 460 nm) spectral regions, coupled to an LG Nexus 5 smartphone. Images of the external surface of the left arm were acquired from a group of young patients (21–31 y) and a group of older patients (50–84 years), and the skin heterogeneities were evaluated using entropy processing. It was observed that blue light provided the most information for identifying skin aging, with a relative entropy difference of approximately 8% [126].

In the acoustic field, sound has been used to visualize tissue through the technique of ultrasonography [127]. Furthermore, other interesting diagnostic techniques that combine light and ultrasound are optoacoustic and photoacoustic [128, 129]. These techniques have not yet been explored by our research group, but excellent groups in Brazil have been dedicated to studying and applying them in the diagnosis of a series of lesions [130].

6 The Role of Certain Scientists in Strengthening Interdisciplinarity in Science

Science is intrinsically multidisciplinary. However, throughout the development of knowledge, sectors were naturally created to facilitate their understanding. In recent decades, it has been proven that the biggest challenges presented to men involve multiple disciplines. Biology is heavily reliant on physics and chemistry. The study of biology today deals more with molecular information than the field of physics itself, and these are only two disciplines focused on different points of view. The need to have multidisciplinary approaches to problems of great relevance is common and already well-established. However, this was not always the case. At the beginning of the twentieth century, the beginning of modern physics, the development of ideas was well divided and persisted for some time. This specialization was an advantage for the advancement of ideas. For example, the development of quantum mechanics would not have advanced so far had the scientists who developed it been engaged in studying biological metabolisms or constructing devices for engineering. Undoubtedly, it would not have advanced as much if there had not been a focus or deep reflection concentrating specifically on atoms and their innermost properties. With the consolidation of certain basic concepts, it is possible to start thinking about connections, from which interdisciplinarity is born. However, traditions sometimes prevent people from taking risks and building bridges that connect different disciplines. Courageous and well-known scientists take risks in establishing these connections. Biophysics is one of the connections that had many contributors, and in Brazil,

Prof. Sérgio Mascarenhas is one of the pioneers who started research groups and approached topics that allowed the existence of interdisciplinarity to become routine [131, 132]. In the 1970s, biophysics was still just a curiosity, but already, there were Brazilians who saw that connecting the principles of physics with biological phenomena would be the future. All the work presented in this article would not exist if the connection between the physical phenomena of molecular excitation, molecular collision, quantum states, and other processes could not be seen within a biological context. The use of ultrasound in a biological environment for the treatment of diseases as important as cancer is a demonstration of the value of interdisciplinarity. Professor Mascarenhas did not invent biophysics, but he strove to apply it in practice in Brazil. We are immensely grateful for this significant effort.

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Declarations

Conflict of Interest The authors declare no competing interests.

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