Laser therapy: physico-chemical properties and assessment of market potential of natural photosensitizers

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Laser therapy has proven to be a method to increase the efficiency, specificity, comfort of dental treatment. Photodynamic therapy (PDT) has been shown to have a strong antimicrobial effect. The objective of this study is to test the market receptivity to the use of a natural experimental photosensitizing agents and their physico-chemical properties in the control of microbial biofilm of the oral cavity. A questionnaire of 11 questions was developed, which was distributed to a sample of 100 subjects for testing in order to complete it. Analyzing the answers, there is a high receptivity of doctors to use photodynamic therapy in all dental branches as antimicrobial therapy in the presence of a natural agent revealer instead of a chemical revelator. Infrared spectroscopy (FTIR) was used as an analytical technique to confirm the structure of the prepared gels, respectively to investigate the interaction between the components of the synthesized samples and also the UV-Vis analysis of the experimental revelators to indicate their maximum absorbance.

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1. Introduction

Laser therapy used in dental practice uses lasers that can be classified according to several criteria: depending on the type of laser used, such as gas laser and solid laser; depending on the tissues where it is used, hard tissue or soft tissue; depending on the wavelength used and, of course, depending on the risk associated with laser therapy.

Absorption requires a chromophore, which absorbs light at a certain wavelength. In soft oral tissues, primary chromophores are represented by melanin, hemoglobin and water, while hard tissue is represented by water and hydroxyapatite. The laser selection procedure used is dependent on the absorption coefficients of primary chromophores at different wavelengths. In dentistry, laser therapy is applicable to both soft tissues and dental hard tissue.

Considering the increasing problem of bacterial resistance to conventional antimicrobial therapy, the use of an alternative bactericidal approach to which bacteria could not develop resistance would be valuable. The rapid increase in antibiotic resistance among pathogenic bacteria may lead to the end of a period that has been taking place over the past 50 years, called "the age of antibiotics". Current treatment for diseases induced by dental plaque in the oral cavity involves, firstly, its mechanical removal from accessible areas and, secondly, the use of topical and / or systemic antimicrobial drugs [1-3].

Photodynamic antimicrobial chemotherapy (PACT), which is characterized by the association of a photosensitizer agent (PS) with a complementary wavelength of light, could be a desired approach.

PACT consists in photosensitizing microbial or cellular components, which are led to an excited state when exposed to a complementary wavelength of light. This change stimulates electrons to move out to higher energy levels, promoting the release of photons. The triplet PS has a sufficiently long lifetime to allow it to undergo chemical reaction. In this excited state, the photosensitizer can interact with molecular oxygen, initiating the formation of highly reactive singlet oxygen (photoprocess

type II). Also, it can interact with other molecules acting as an electron receptor, which results in the production of hydroxyl and other organic radicals (photoprocess type I). The products of these photochemical reactions may irreversibly affect the cell metabolic activities as well as damage essential cell components, such as the cytoplasmic membrane, resulting in bacterial death.

The result of the interaction between the light source and the bacterial cell depends on the wavelength of light, the potency, the irradiation time, the diameter of the probe, the light emission mode (continuous or pulsed), and if the irradiation is focused or defocused [4-6].

Since the beginning of the last century, it has been known that certain micro-organisms can be killed by combining non-toxic colorants, known as photosensitizers, in the presence of innocuous visible light in vitro [7]. Over the years, there have been additional reports of bacteria, fungi and viruses that have been killed or inactivated by various combinations of PS and light. Antimicrobial PDT (α-PDT) is a localized, non-thermal and non-invasive antimicrobial method of reducing bacterial contamination in oral infections. It has a large microbicide effect, in addition to better access to places inaccessible to conventional therapy. Several studies have shown that PDT has a strong effect on a large number of gram positive and gram negative oral bacteria, using various photosensitizers and light sources [8-13].

All studies that examined the killing of antibiotic resistant bacteria by combining PS and light, called photodynamic inactivation (PDI), have shown that they are as sensitive as their counterparts. Examples of such relatively novel therapies are bacteriophages, natural or synthetic antimicrobial peptides and PDT. Since the early days of the PDT, at the beginning of the last century, it is known that certain microorganisms can be killed by combining colorants and light in vitro [2,14,15].

In order to be suitable for antimicrobial PDT, the ideal PS should possess low levels of dark toxicity and the presence of absorption bands in the so-called optical window (600–900 nm) for sufficient tissue penetration of light. Moreover, the PS should have relatively high absorption bands (>20,000–200,000 $M^{-1}cm^{-1}$), that in turn will diminish the dose needed to deliver the desired effect. The PS should also have a high yield of excited electronic triplet state and of singlet oxygen [16].

The idea of using a natural compound as a PS, inherently contains possible contradictions. However, there are several isolated natural compounds that have been extensively explored as PS. Laser therapy is a multidisciplinary field involving chemists, physicists, biologists, engineers and doctors. Chemists, of course, are constantly trying to design, synthesize, purify, and characterize new compounds that can be used as PS. The emergence of the revolution in nanotechnology has had a major impact on PDT and expects to further influence this field. [17-20]

The objective of this study is to test the market receptivity to the use of a natural experimental photosensitizing agent and their physico-chemical properties in the control of microbial biofilm of the oral cavity. The opportunity to launch on market an autochthon revelator used as a photosensitising agent in the antimicrobial photodynamic therapy was also followed, as well as the receptivity of doctors in connection with the use of a natural revelator used in microbial biofilm control of the oral cavity [3,12].

Infrared spectroscopy was used as an analytical technique to confirm the structure of the prepared gels, respectively to investigate the interaction between the components of the synthesized samples and also the UV-Vis analysis of the experimental revelators to indicate their maximum absorbance [3,13].

2. Materials and methods

2.1. The preparation of the gel

The gels were prepared from a mixture of gelatin: glycerol (Sigma-Aldrich) in a weight ratio of 1:1 and 60 ml Kaqun® (Harghita, Romania) water, using the following procedure: gelatin, glycerol with 0.015 % salicylic acid solution were added to the Kaqun water. The gel formed, was divided into equal parts, in which it was added Oregano essential oil (Young Living, USA) and curcumin extract.

2.2. Physical and chemical properties of the new photosensitizers

Determination of the physico-chemical characteristics of the product obtained from the homologated laboratory technology was made by analyzing FT-IR and UV-vis. Infrared spectroscopy FT-IR (JASCO 610) was used as an analytical technique to confirm the structure of the prepared gels, respectively to investigate the interaction between the components of the synthesized samples. Gel spectra were recorded: Gel - Initially; Gel-O (essential oil of oregano); Gel-O-Cu (oregano and curcumin) and Gel-Cu (curcumin).

UV-Vis analysis of the experimental revelators was done in order to determine their maximum absorbance with Lambda 25 (PerkinElmer Singapore) spectrophotometer.

2.3. Research coverage

Due to the specificity of the products that generated this study and the fact that the first targeted users will be dentists, teachers, private and state doctors, the researched market was represented by dentists in the Romanian Universities of Medicine and Pharmacy, as well as private and state dental practitioners from other countries.

2.4. Research tools

The tool used to collect the data was the questionnaire. Starting from the assumptions made, a questionnaire was prepared, which was distributed to a

sample of 100 subjects for testing in order to complete it. There were 11 questions, choosing carefully the specialized language, the type and order of the questions. It has also been called upon to question dentists to make suggestions and recommendations on this type of material.

2.5. Sampling

The size of the sample was set at 100 private and state dentists in both Romania and other countries.

We consider that for this type of study the size of the sample was sufficient due to the size of the market on which the research is carried out and we consider the units selected as representative for obtaining the necessary information.

2.6. Data collection

The questionnaires were sent online, accompanied by an appendix explaining the importance of this study for the development of this type of material, while also providing dentists with the confidentiality of the data provided. The percentage of responses received was 82%, which demonstrates the importance given by subjects to the issues addressed and has led to obtaining sufficient information to complete the study.

3. Results and discussion

Since the support of decision-makers is absolutely necessary in the process of implementing innovative procedures and methods in the activity of any entity, the research mainly reflects the dentist's opinion about the need for a natural photosensitive agent used in antimicrobial photodynamic therapy and the opening to a new therapeutic approach to control biofilm in the oral cavity.

Of the respondents, the majority are female (85.7%) and fall into the age group of 31-40 years (48.2%), respectively 21-30 years (35.7%) (Fig. 1).

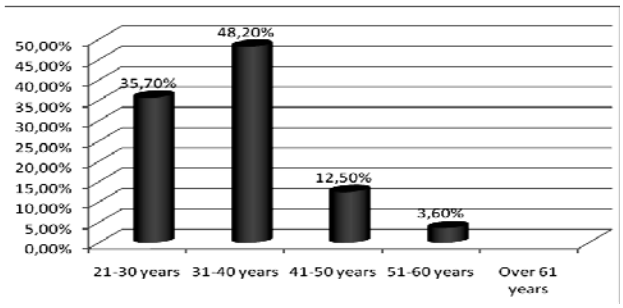

Fig. 1. Age category to which respondents belong

Asked about the way to highlight the bacterial plaque they use in the dental office, most doctors responded that they were using bacterial plaque revealers (64.3%), while 28.6% said they were using other dyes (Fig. 2).

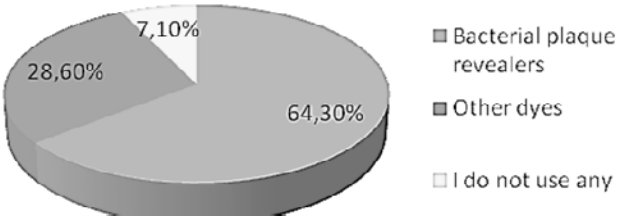

Fig. 2. Which way to highlight the bacterial plaque use the dentists in their dental office

In terms of non-invasive and minimally invasive prophylactic treatments used in the dental office, 96.4% referred to ultrasound scaling, 89.3% to oral hygiene, 78.6% to airflow and dental scaling, 58, 9% in topical fluoridation and 17.9% responded to other treatments (Fig. 3).

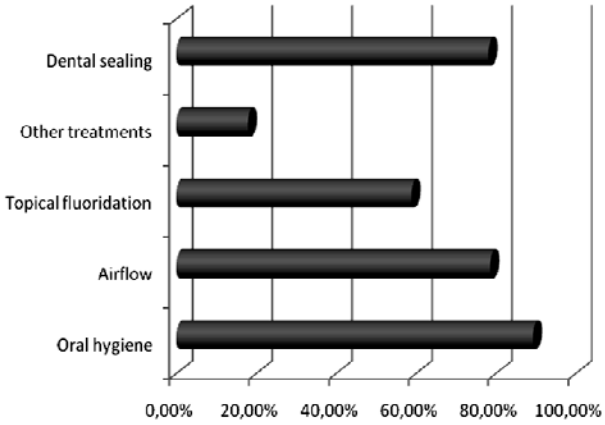

Fig. 3. Non-invasive and minimally invasive prophylactic treatments used in the dental office

As antimicrobial therapy used in the dental office, 94.6% of the doctors responded that they use professional hygiene, 57.1% use topical drug therapy, 21.4% use systemic drug therapy, and 1.8% use chlorhexidine (Fig. 4).

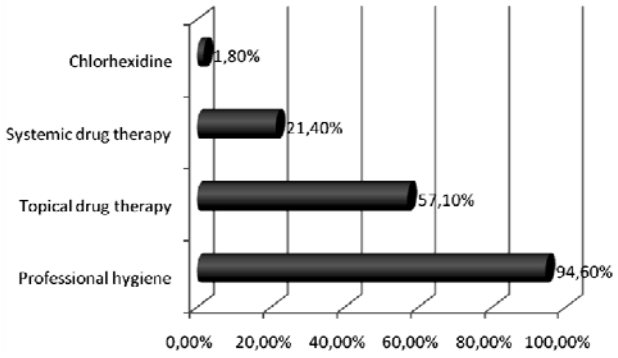

Fig. 4. Antimicrobial therapy used in dental offices

In terms of photodynamic therapy, 64.3% of doctors claim that they do not have information about this procedure (Fig. 5), but 94.6% of physicians would be interested in using photodynamic therapy in the presence of a natural revealer (Fig. 6), which shows the increased interest of doctors in this procedure.

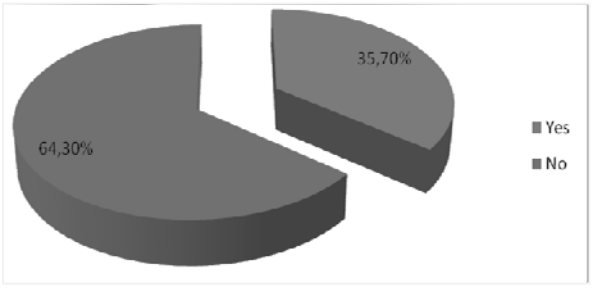

Fig. 5. Percentage of doctors holding information about photodynamic therapy

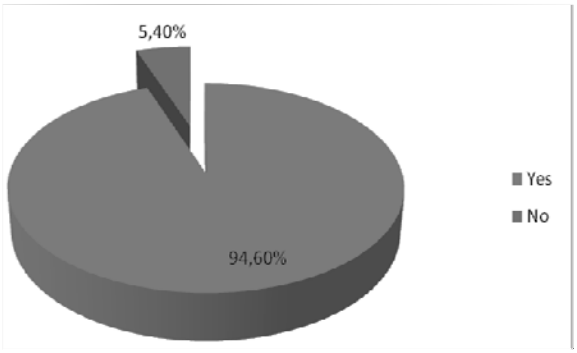

Fig. 6. Percentage of doctors who would be interested in using photodynamic therapy in the presence of a natural agent revealer

The interest of dentists for a dental material with natural agents rather than a chemical material results from the very high percentage (92.9%) of doctors who answered the question "What type of revelator would you like to use in photodynamic therapy? "(Fig. 7).

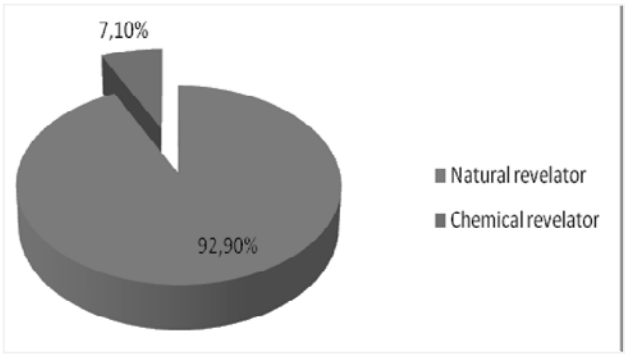

Fig .7. What kind of revelator would want to use dentists in the photodynamic therapies

Asking if they have a Laser device in the dental office, 91.1% of the doctors responded negatively (Figure 8), but those who responded affirmatively said they had a diode laser.

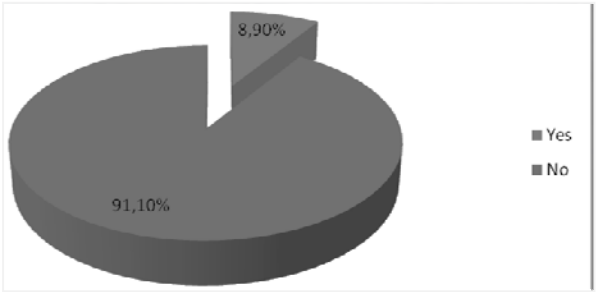

Fig. 8. Percentage of doctors holding a laser device in the dental office

The physicians' responses to the diseases for which they use or would like to use this therapy are in line with research in recent years on areas of use of photodynamic therapy: as antimicrobial therapy in odontal, endodontic, surgical, periodontal, dental aesthetic treatments.

As shown in Fig. 9, gelatin based gel is characterized by absorption bands of amide I (1640-1644 cm^{-1}), the amide III (1236-1241 cm^{-1}) characteristic absorption bands in the form of collagen disordered protein chains, suggesting that gelatin retains its disordered structure even after the gelation process.

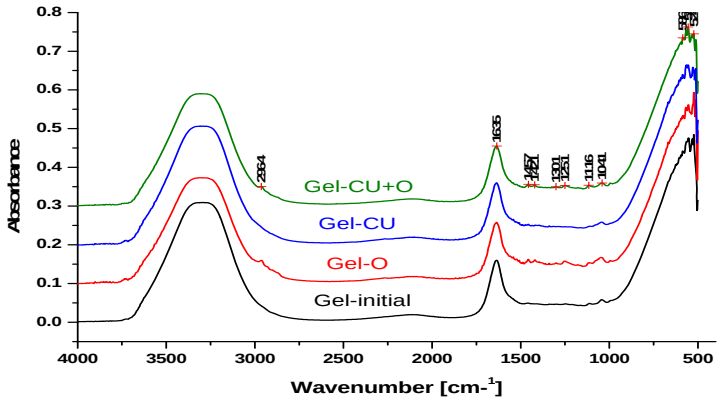

Fig. 9. IR absorption spectra for gel samples containing essential oil of oregano (Gel-O) and curcumin extract (Gel-Cu)

It is noted (Fig. 9) the absorption band characteristic of the amide group I at 3100 -3500 cm^{-1} to the control sample (initial gel) and the samples with oregano essential oil and curcuma extract and the vibrations of water molecules from the gel. The band around 2964 $cm^{-1}$from IR spectra of the gel with oregano essential oil and oregano/curcuma, can be attributed to the aromatic C-H stretching vibration, while the peaks at 1457 cm^{-1} and 1421 cm^{-1} can be attributed to the C-C stretch of the aromatic ring of carvacrol, the main oregano component. The characteristic absorption of the C-O stretching vibration appears at 1251 cm^{-1}. The increase in the intensity of the absorption band at 1041 cm^{-1} attributed to the C-OH binding strength of glycerin in the spectrum of salicylate crosslinked gels with oregano essential oil, relative to the band of the control sample spectrum, may be due to the more intense vibration of the functional groups -OH from the gel structure.

The FT-IR spectrum of the hydrogel loaded with essential oil of oregano and curcumin extract shows that no new bands were formed, meaning that there are no chemical interactions between the hydrogel matrix, salicylic acid, and curcumin or oregano oil. Physical interaction thanking places are more beneficial in our case than chemical interaction, because antibacterial activity can be inhibited following chemical interactions. The concentration of essential oil of oregano and curcumin extract is low in these gels, and the quantity of water content is high, so that the identification of peaks for existing compounds is difficult due to the vibrations of water molecules that superpose over the vibrations of gelatin.

The comparative analysis by UV-Vis spectrometry of the studied oils shows the following absorption spectra (Fig. 10).

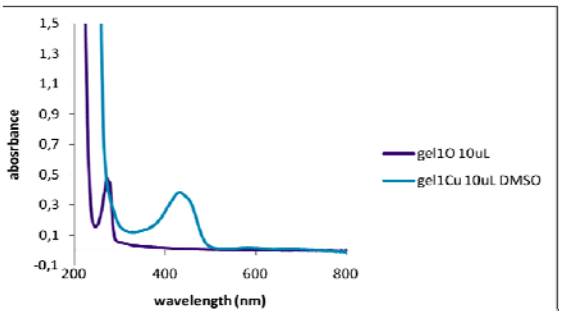

Fig. 10. UV-Vis spectra of the studied gels with oregano (gel O) and curcumin (gel Cu)

All samples show absorbance, but in the UV field they are distinguished by a well defined 276 nm shoulder of oregano oil and at 440 nm the curcumin based gel.

The low-level laser therapy (LLLT) has gained in the present a very large spreading between practicing physicians. Low-level laser therapy at adequate wavelength, intensity, and dose can accelerate tissue repairing. Laser treatment facilitates the obtaining of better results for most of the treated patients. Also laser treatment shows better results as the classical treatment in most affections and no side effects were observed [21].

Photodynamic therapy, an alternative to antibiotics for the treatment of bacterial infections, uses photosensitizing substances activated by light of an appropriate wavelength. Activation of photosensitizers leads to the production of reactive oxygen species (ROS), which is lethal to the target cells. Photodynamic therapy has been studied as a promising approach to the eradication of oral pathogens that cause diseases such as periodontitis, perimplantitis or dental cavities, so PDT can be an appropriate antimicrobial approach that could eradicate biofilm and antimicrobial resistance [1,5,22].

Nowadays, laser treatments alone or PDT in combination with photosensitizers have become popular as new therapeutically techniques in dentistry and are used in a large number of dental procedures, including the treatment of periodontal diseases, peri-implantitis infections and endodontic ones. Given the non-invasive and local nature of lasers, the incidence of numerous side

effects associated with the use of antibiotics, including gastrointestinal mucosal lesions and the appearance of drug allergies, is unlikely [23,24].

At the moment, there is considerable interest in using antimicrobial agents applied locally in the treatment of periodontitis. A major advantage of this approach compared to systemic administration of such agents is that it minimizes the disruption of normal micro flora in other parts of the body, thus helping to avoid opportunistic infections [25,26].

Photosensitizing substances can exist naturally in cells and tissues (flavine, pyridine, bilirubine, etc) or can be administered under control as in photodynamic therapy. Until now many substances have been tested both in vitro and in vivo none being perfect. Nowadays research is aimed at discovering new substances [27].

An ideal photosensitizer must be biologically stable, photochemically efficient, selectively retained in the target tissue and must have minimal toxicity in other areas than the target [10].

Special effort is being made to develop new sensitizers. In particular, new compounds are needed to absorb light at longer wavelengths to aid in tissue penetration, to greater PDT efficiency, selective tissue localization and self-limiting soft tissue photosensitivity. Many other sensitizers have been described for this purpose [28,29].

4. Conclusions

Analyzed by UV-Vis spectrometry, oregano and curcumin essential oils in the study have UV absorption, which proves their applicability as natural photosensitizers in low-level laser therapy. By infrared spectroscopy it has been emphasized that there were no interactions between oregano and curcumin and gelatin matrix. Analyzing the answers to the questionnaires distributed among dentists, there is a high receptivity of doctors to use photodynamic therapy in all dental branches as antimicrobial therapy in the presence of a natural agent revealer. Dentists have expressed interest in a dental material with natural agents used as a revelator in photodynamic therapy, to the detriment of a chemical revelator, therefore permanent collaboration between chemist researchers doing these materials and dentists testing them and having some expectations about their quality is needed.

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