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Noninvasive Digital Method for Determining Inflammation after Dental Implantation

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Abstract: This study shows that the luminescent diagnostic of oral fluid allows the determination of the severity of inflammatory markers after implantation. The noninvasive diagnostic method, which is used, allows the rapid detection of the stages of development of the inflammatory process after intraosseous implantation and prevents the development of complications in the postoperative period.

Keywords: implantation; inflammation; fluorescent diagnostics; protoporphyrin IX; oral fluid

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Citation: Prikule, D.V.; Kukushkin, V.I.; Prikuls, V.F. Noninvasive Digital Method for Determining Inflammation after Dental Implantation. *Biophysica* **2022**, *2*, 412–416. https://doi.org/10.3390/biophysica2040036

Academic Editor: Danilo Milardi

Received: 15 September 2022
Accepted: 26 October 2022
Published: 1 November 2022

1. Introduction

Dental implantation is one of the most rapidly developing areas of modern dentistry [1,2]. It allows for the replacement of lost teeth without the preparation of adjacent teeth. The fundamental process after the implantation operation is the osseointegration of the implant in the jawbone tissue. However, the effectiveness of osseointegration can be significantly reduced as a result of the progression of symptoms of inflammation in the postoperative field after implantation [2–4]. Digital diagnostic methods (orthopantomogram (OPG), multi-slice computed tomography (MSCT), and cone beam computed tomography (CBCT)) are widely used to determine the current situation in the bone tissue after the implantation, which makes it possible to identify the pathological process in the studied tissues [5–8]. However, it should be taken into account that, due to the use of these methods, it is possible to determine the already existing inflammation and the task of the early detection of changes in the homeostasis of the body, which can lead to subsequent inflammation in the implant area, today have no solution in world medical practice. The degree of inflammation in tissues in the postoperative field area can also be determined using the periodontal index [9] and papilla-marginal-alveolar index [10] in the early stages and further [11,12]. It is possible to determine the degree of hydration of the extracellular environment of tissues using a bioimpedance analyzer, impaired blood supply in tissues by the laser-Doppler flowmeter, and capillaroscopy [13–17]. However, these methods are significantly laborious and difficult to carry out in the postoperative period.

At the same time, modern methods for diagnosing the state of tissues using optical spectroscopy are actively used in other areas of medicine and make it possible to identify various pathological processes in the tissues and biological fluids by monitoring biochemical changes happening in them [18–24]. Optical spectroscopy methods can quantify changes in various metabolites (glucose, cholesterol, phenylalanine, glutamic acid et al.) [25] and fluorochromes of the tissues and bioliquids (nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH)), tryptophan, carotenoids, elastin, collagen, flavins, porphyrins) [26].

It Is known that fluorescent porphyrins (protoporphyrin-IX, uroporphyrin, coproporphyrin) interact effectively with laser radiation and are excited to high-energy states. As a result of such decomposition to the porphyrins, singlet oxygen is formed. Porphyrin-induced oxidative stress is thought to be the main mechanism of tissue damage [27]. In addition, as a result of a number of studies, it was found that porphyrins were markers of inflammatory processes in the human body, as well as in situations that are characterized by a state of increased stress—that is a violation of the individual's body homeostasis [28]. The possibility of diagnosing inflammatory processes by determining the intensity of the luminescence of porphyrins contained in the oral fluid, and the possibility of the large-scale use of modern ergonomic optical spectrometers for detecting luminescence signals, would make it possible to detect early signs of inflammation after the implantation process.

Thus, the development of a method for the non-invasive diagnosis of inflammation after implantation (in the early stages) and the determination of the severity, duration, and progression of the inflammatory processes are extremely relevant problems. The aim of our study is to create a non-invasive method for dynamic control of the degree of inflammatory reactions in the postoperative period, with the results recorded in digital form.

2. Materials and Methods

2.1. Luminescence Spectroscopy

The study of the oral fluid was carried out on a luminescent spectrometer, "EnSpectr M" (MEDANALIS, Moscow, Russia), with a laser wavelength of 405 nm. The spectral range of the device was 420–700 nm. When measuring the luminescence spectra, a special nozzle for liquid samples was used. The special cylindrical glass vials with a volume of 1.5 mL (Akvilon, Moscow, Russia) with the sample under study were inserted into vials. A laser beam with a diameter of 200 μm was focused on the center of the vial with the sample. The laser radiation power was 20 mW, and the exposure time for recording spectra was 1 s. The registration of the spectra was carried out within a few minutes after obtaining the biomaterial. The wavelength of the laser radiation and the spectral range of registration were chosen based on the conditions of excitation and the position of the luminescence peak of protoporphyrin IX. The selected radiation power density made it possible to perform measurements in the express mode and did not lead to the photobleaching of the test sample.

2.2. Patient Groups and Samples

An unstimulated oral fluid was collected from 25 patients (14 females and 11 males) using a pipette from the sublingual region into special disposable plastic containers (Eppendorf tubes, 1.5 mL), mainly in the daytime. The patients' diet did not change. Teeth brushing was carried out as standard twice a day and was not performed specifically before the diagnostic procedure. The average age of the examined patients was 39 years, and the age range was from 32 to 47 years.

The following inclusion criteria were used throughout the study:

- Placement from 1 to 3 intraosseous implants in the mandible;
- Men and women from 32 to 47 years of age;
- All patients were non-smokers;
- The absence of chronic diseases in patients at the stage of decompensation, foci of chronic infection in the oral cavity, and pregnancy.

The non-inclusion criteria were:

- Immediate implantation after removal of the corresponding teeth;
- Guided bone regeneration;
- Bad level of oral hygiene (more than 3.1 points of index values in the simplified oral hygiene index (OHI-S)).

Biomaterial sampling was carried out before and after implantation, as well as on the following days: days 1, days 3–4, and days 7–10 after surgery. The period after

implantation (on the day of the operation) is a period of time 30–60 min after the end of the operation. Prior to implantation, patients were trained in oral hygiene procedures, and subsequently, all patients were assessed for their level of oral hygiene using OHI-S (Greene, J.C., Vermillion, J.R., 1964) in order to exclude the development of inflammation due to microbial plaque. The vestibular surfaces of teeth 1.6, 1.1, 2.6, and 3.1 and the lingual surfaces of teeth 3.6 and 4.6 were stained using a President Plaque test (President Florence, Italy). The data were interpreted using codes and criteria for assessing plaque [29].

3. Results

The main fluorophores emitted in the visible spectral range are lipopigments, flavins, and porphyrins [30]. The lipopigments have an absorption maximum of about 340 nm and an emission maximum of about 545 nm. Flavins are characterized by their strong absorption over a wide spectral range from 200 nm to 500 nm, with strong absorption maxima at 220 and 260 nm and a weaker absorption at 380 and 460 nm. The maximum emission of flavins is 555 nm [31]. Porphyrins are characterized by their broad absorption in the region of 300–470 nm with a maximum of 400 nm. The emission of porphyrins has a maximum of around 630 nm [32]. Based on these data, a source of laser radiation with a wavelength of 405 nm and a spectrometer with a wide spectral range of 420–700 nm was selected for the purpose of the highly sensitive determination of inflammatory markers (porphyrins).

The revealed results of the study of the oral fluid at the control stages indicated an increase in the level of Protoporphyrin IX up to 1697 rel. units (S = 87) in patients before implantation (Figure 1). We associate the dynamics of the increase in this indicator with the presence of an increased stress level noted in the preoperative period. At the same time, during the control immediately after the end of the surgical stage, we recorded an increase in the biomarker indicated above by 3785 rel. units (S = 143). However, when controlled after 1, 3–4, and 7–10 days, we observed a trend towards a decrease in the level of Protoporphyrin IX by, respectively, 2234 rel. units (S = 131), 1812 rel. units (S = 129) and 604 rel. units (S = 97). The conditions for recording the photoluminescence spectra were the same in the presented pictures; therefore, the relative intensity of the 630 + 10 nm line of protoporphyrin IX indicates the severity of inflammation after the implantation process. Immediately after implantation, the luminescence intensity increased by more than two times and then gradually decreased over time (more than six times over 10 days compared to the maximum value). At all stages of control, we observed a good level of oral hygiene according to the OHI-S index. Thus, due to the maintained level of oral hygiene at the control stages and further, we excluded the microbial component from the possible dynamics of the inflammatory response.

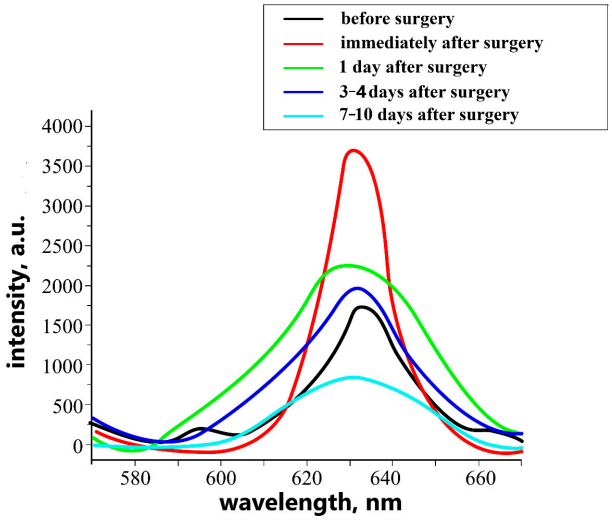

Figure 1. Dependence of the luminescence intensity of Protoporphyrin IX on the dynamics of the development of inflammatory processes as a result of implantation.

4. Conclusions

The use of the luminescent diagnostics method for the oral fluid using the hardware-software complex "EnSpectr M" with a laser radiation wavelength of 405 nm allows for the determination of the severity of inflammation markers (Protoporphyrin IX), taking into account the individualization of the clinical case. For the first time, the diagnostic criteria allowed the possibility of predicting the development of an inflammatory process after intraosseous implantation and the ability to plan an individualized complex to prevent the development of complications after dental implantation.

When comparing the method of luminescence spectroscopy with index methods (PI, PMA), it was possible to draw conclusions from numerical values, while the index methods were accompanied by an approximate visual assessment. When comparing luminescent diagnostics with a bioimpedance analyzer, laser-Doppler flowmetry capillaroscopy, the advantage was the absence of the need for contact with the tissues of the postoperative area and the possibility of the specific detection of an inflammation marker.

Author Contributions: Conceptualization, D.V.P., V.I.K. and V.F.P.; methodology, D.V.P. and V.I.K.; formal analysis, D.V.P. and V.I.K.; writing—original draft preparation, D.V.P., V.I.K. and V.F.P.; writing—review and editing, D.V.P., V.I.K. and V.F.P.; project administration, V.I.K. and V.F.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to the Osipyan Institute of Solid State Physics of the Russian Academy of Science (ISSP RAS).

Conflicts of Interest: The authors declare no conflict of interest.

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