

An interview with

Gustavo Sivieri-Araujo

- » Graduate in Dentistry from the Universidade de Uberaba – UNIUBE
- » Update course in Endodontics at the Faculdade de Odontologia de Araraquara - FOAr-UNESP;
- » Specialist in Endodontics from the Faculdade de Odontologia de Araraquara - FOAr-UNESP;
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- » Post-Doctorate in Physics and Materials Science from the Instituto de Física de São Carlos - IFSC-USP;
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Interviewer

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To begin this interview, tell us about your academic background and the line of research in which you work.

Mineiro from the city of Uberaba-MG, I was born, raised, and studied in this city. Since the beginning of my undergraduate studies, I have always had an attraction to an academic career, I have always been determined to do something that had some connection to what I wanted to become in the future. I was an intern in the Pathology discipline and worked as a monitor in Endodontics I and II at the University of Uberaba - UNIUBE.

As soon as I graduated (1996), I was a dental surgeon at the Naval Hospital in Brasilia of the Navy Ministry for 18 months. After this period, I was sure that my future was not in the military career.

After that, I took an update course (1997) and a specialization course (1998-1999) in Endodontics at the Araraquara Dental School - FOAr-UNESP.

I worked as a clinician in the office for almost 7 years and in the UBS of the Uberaba City Hall for almost 2 years. But the certainty that my path would be to return to my studies toward graduate school was increasingly evident. During this period I was also an intern for the Histology course at UNIUBE. I also took a refresher course in Pathology at the Medical School of the Federal University of the Triângulo Mineiro - FM-UFTM.

With much effort and persistence, I was approved for the master's course (2004) in Endodontics at the Araraquara Dental School - FOAr-UNESP. I moved to the city of Araraquara and was faithfully dedicating myself to the course. This was the fact that allowed me to be rewarded with the change of course level from Master's to PhD, moving straight from one to the other in early 2005. Time passed and when I realized, it was already the defense of my doctoral thesis (2008). I defended my thesis, whose theme was "Low intensity laser in tissue response to Endofill endodontic cement", under the supervision of Prof. Dr. Fábio L. C. V. Berbert, with the support of the CAPES scholarship.

After that, I was approved in the contest for substitute professor of Integrated Clinic I and II of the Dentistry Department of the Health Sciences School of the Federal University of Brasilia - UnB for almost 2 years (2008-2009). Even before finishing my contract as substitute professor at UnB, I was heading in search of a post-doctorate. I looked for

Prof. Dr. Vanderlei S. Bagnato from the São Carlos Physics Institute, University of São Paulo - IFSC-USP to work with photodynamic therapy (due to the line of research I had worked on during my doctorate). I moved to the city of São Carlos-SP (2010 and early 2011) and we developed the project with the support of a CNPq grant.

Concomitantly to everything that was happening, I found out about the edict for substitute professor in Endodontics at the Araçatuba Dental School - FOA-UNESP. This became irresistible and I was approved in this contest, staying for a period of 1 year living in Araçatuba-SP (2011).

In late 2011, I was invited by Prof. Dr. Joao E. Gomes-Filho to do a Research Project to perform another postdoctoral fellowship, now with FAPESP scholarship for the years 2012 to 2014 and work with photodynamic therapy.

In September 2013, prof. Dr. Mauro J. Nery retires from the discipline by Compulsory retirement and, in October 2013, prof. Dr. Pedro F. E. Bernabé retires, due to winning the Election for Mayor of the city of Birigui-SP. Therefore, I knew that soon there would be a contest (2 vacancies) in the discipline of Endodontics FOA-UNESP. At the same time that I was happy for the possible opportunity, I was also apprehensive, because if I was not approved, the uncertainty could catch me again.

But, thank God, on April 8, 2014, I was approved in that contest for the position of Assistant Professor. So I took office exactly one month later, on May 8, 2014.

We asked for the termination of my FAPESP post-doctoral fellowship, with the commitment of finishing the Research Project, so that I could assume the contract as a full professor of the Endodontics discipline at FOA-UNESP. I am until the present moment in full activities with this renowned discipline of Endodontics at FOA-UNESP, which started with Drs. Roberto Holland and Valdir de Sousa.

Seeking to continue the academic career progression in this year of 2020, I would take the free-doctoral competition in Endodontics on May 22 and 23, 2020, and become an associate professor. However, due to the COVID-19 pandemic, all free-doctoral and full professor competitions at UNESP have been temporarily suspended. I hope that soon I will be able to take another important step in my academic career.

What is photodynamic therapy and what is its mechanism of action?

Photodynamic therapy (PDT) is a set of physical, chemical, and biological procedures that, when associated, form a photobiochemical reaction to destroy damaged tissue and/or inactivate microorganisms (target cells).^{1,2}

The mechanism of action occurs after administering an exogenous photosensitizer, which, in the presence of oxygen, is activated by a light (laser or LED) that has a specific wavelength for each type of photosensitizer used. A synergism occurs, leading to oxidative stress, and this interaction produces highly reactive oxygen species (free radicals and especially singlet oxygen) that instantly eliminate pathogenic cells exclusively, because they promote specific cell death (by cell necrosis, apoptosis, or autophagy) of the target cell.¹⁻³

The reactions of photodynamic therapy on the target cell and/or microorganisms occur at the molecular level. The Type I reaction can raise hydroxyl radicals that react with molecules to form hydrogen peroxide in situ. The removal of hydrogen from the phospholipid molecules of the bacterial cytoplasmic membrane alters membrane permeability and integrity.^{2,4}

In the Type II reaction, the photosensitizer transfers its energy to oxygen, which forms the singlet oxygen. The short lifetime of the singlet oxygen leads to a response at the target cell site. Singlet oxygen reacts with cell walls, nucleic acids, peptides, and molecules involved in the structure of the cell wall.^{4,5}

What types of light source can be employed in photodynamic therapy?

Photodynamic therapy employs a light source of specific wavelength to the photosensitizer, such as a low-intensity laser or LED.⁶ Regardless of the use of low intensity laser or LED, for photodynamic therapy to be applied to endodontics, a thin, disposable, flexible optical fiber must be connected to the device so that it can be introduced inside the root canals.

Lasers have characteristics such as monochromaticity, divergent and coherent light, ultra-short pulses, wavelength adjustments. LEDs are light-emitting diodes, with a low thermal component and a relatively narrow spectral band, which is produced by the energetic interactions of the electron.^{6,7}

What are photosensitizers?

Photosensitizers are a set of molecules that interact with light and give rise to highly reactive oxygen species, such as free radicals and singlet oxygen.⁸

Its action in conjunction with a light of specific wavelength (absorption spectrum of the photosensitizer) results in generally irreversible photodynamic effects, with destruction of target cells or pathogenic tissues (specific sites).^{8,9}

After absorbing a photon, the photosensitizer molecule temporarily switches from the fundamental state to the stimulated (singlet) state. On the way back to the fundamental state, it emits photons through processes called fluorescence.^{8,9}

Photosensitizers need to have biological selectivity and stability, be photochemically efficient, and offer low toxicity to healthy tissues. The lower the toxicity of the photosensitizers with the absorption bands and the closer to the wavelength used, the greater the efficiency of the desired photodynamic effect, without causing damage to adjacent tissues.⁸⁻¹⁰

What types of photosensitizers are used in photodynamic therapy?

There are several types of photosensitizers, each of which has a specific wavelength for each type to be applied. It is necessary to know the classes of photosensitizers available to know how to apply them to each clinical indication.¹⁰

We can mention the following classes of photosensitizers: porphyrins, chlorines, phthalocyanines, phenothiazines (methylene blue, toluidine blue, acridine orange), xanthenes (rose bengal), phenolic compounds (curcumin). Photosensitizers can be found commercially in liquid, gel or ointment form.¹¹

In dentistry, in general, xenothiazines (methylene blue) are most frequently used, at a concentration of 50 mg/L (0.005%) or 100 mg/L (0.01%), which are activated by a low intensity red laser ($\lambda=660$ nm).¹²

What are the indications and general applications of photodynamic therapy?

Photodynamic therapy is used in general in the health field (medicine, dentistry, veterinary medicine, and physiotherapy) to treat oncological diseases and to combat different species of microorganisms, acting to control infections against bacteria, viruses, and fungi.¹³

Photodynamic therapy has also been applied to decontaminate blood, food, surfaces, and to detoxify water.¹⁴

Currently, photodynamic therapy has shown good results, as a good alternative in infection control with coronavirus, the cause of COVID-19.¹⁵

What are the indications and applications of photodynamic therapy in dentistry?

In dentistry, photodynamic therapy can be used in almost all clinical specialties (Periodontics, Endodontics, Implant Dentistry, Dentistry, Prosthodontics, Pediatric Dentistry, Surgery, and Stomatology) to fight infections of different types of microorganisms.^{16,17}

Photodynamic therapy can also be used in the treatment of oncological lesions of the oral cavity (Pathology and Stomatology).¹⁸

What are the indications and applications of photodynamic therapy in Endodontics?

In order to promote microbial reduction and potentiate the whole endodontic strategy already established, we can employ photodynamic therapy as an adjuvant in the following clinical cases: endodontic treatment of necropulpectomies; retreatments with or without periapical lesions; endo-Periodontal lesions; parendodontic surgeries; decontamination of the prosthetic space before cementation of intracanal pins; and as an aid in the decontamination of cases of fractured instruments in which their removal was not successful in teeth with necrotic dental Pulp.¹⁹

In the case of biopulpectomy treatment, it is not necessary to perform photodynamic therapy, because the root canal system is free of microorganisms; therefore, there is absence of endodontic infection in these specific cases.^{19,20}

It is worth remembering that in recent years, strong evidence has shown the interrelation of endodontic infections with systemic diseases. In these cases, it would be interesting to associate photodynamic therapy, in order to potentiate the fight against microorganisms that cause endodontic infections.

What are the benefits of photodynamic therapy in Endodontics?

We can cite the following benefits of photodynamic therapy in Endodontics:

Primary Effect: Reduced Microbial Load; does not promote microbial resistance; has no side effects; can be applied several times; easy-to-apply; painless; and atraumatic.^{19,21}

Side Effects: Laser therapy/led therapy benefits - photobiostimulation and photobiomodulation, important for tissue repair; analgesic effects (24h to 72h), relieving pain; and anti-inflammatory effects, as it modulates the post-endodontic treatment inflammatory process.^{19,21}

Tell us about Photodynamic Therapy, antimicrobial activity and biological properties (cytotoxicity and biocompatibility) in Endodontics

The literature has shown that methylene blue and curcumin photosensitizers have promising effects regarding antimicrobial activity, capable of inactivating gram-positive and gram-negative bacteria,²²⁻²⁴ as well as a positive effect in inactivating *Enterococcus faecalis*.^{25,26}

Regarding the biological properties, our research group carried out studies to evaluate the curcumin photosensitizer at a concentration of 500 mg/L in cytotoxicity tests (to evaluate cell viability in L-929 cells)²⁷ and biocompatibility (to evaluate biocompatibility in subcutaneous tissue of rats)²⁸, compared to saline (0.9%) and sodium hypochlorite solutions (2.5% and 5%). Photodynamic therapy with the photosensitizer curcumin 500 mg/L showed cell viability and biocompatibility similar to 0.9% saline solution. On the other hand, sodium hypochlorite solutions (2.5% and 5%) were shown to be toxic to L-929 cells, as well as not biocompatible with the tissue response of rats.

What about the photodynamic therapy and the mechanical properties (Martens hardness, modulus of elasticity and bond strength) in relation to the intraradicular dentin?

Subsequently, and from the favorable biological results (cytotoxicity and biocompatibility), and also due to the inexistence of results in the literature of tests of the mechanical properties on this curcumin photosensitizer, our research group started to evaluate these properties of photodynamic therapy in relation to the intradicular dentin and also the bond strength of glass fiber pins to intradicular dentin at concentrations of 500 mg/L and 1000 mg/L.

However, when searching the databases, we found that the literature lacks results of tests of the mechanical properties also with methylene blue photosensitizer (Martens hardness and modulus of elasticity tests) on intradicular dentin, as well as the test of bond strength of glass fiber pins to this intradicular dentin (push-out test) in concentrations of methylene blue found commercially of 50 mg/L (0.005%) and 100 mg/L (0.01%), respectively. The results of these studies demonstrated that photosensitizers can influence the mechanical properties of the intradicular dentin and the bond strength of glass fiber pins depending on the type and concentration of the photosensitizers used.²⁹ The curcumin photosensitizer altered the mechanical properties of intradicular dentin at concentrations of 500 mg/L and 1000 mg/L.³⁰ The methylene blue photosensitizer at a concentration of 50 mg/L did not alter the mechanical properties of the intradicular dentin and/or bond strength.³¹

What is the clinical protocol for the application of photodynamic therapy in Endodontics?

To perform photodynamic therapy in Endodontics, it is necessary to perform the entire sequence of necropulpectomy endodontic treatment (biomechanical preparation, root canal irrigation, cleaning of the foramen, and use of EDTA).

From this step on, the photodynamic therapy is constituted. After the photodynamic therapy, the intracanal calcium hydroxide medication is used.

The step-by-step clinical protocol for photodynamic therapy can be described as follows:^{22-24,29-31}

» Insert the photosensitizer, e.g. methylene blue (0.005% or 0.01%), into the root canals and leave for 3 minutes (pre-irradiation period). Avoid extravasation beyond the apical foramen. If you wish to potentiate the action of the photosensitizer, ultrasonic agitation (without irrigation) for 20 to 60 seconds can be done at this time, with fine ultrasonic tips, so that the photosensitizer can better penetrate the root canal system;

» Activate the red laser for 60 (Energy=6 J, Dose=200 J/cm²) to 90 seconds (Energy=9 J, Dose= 300J/cm²), using helical movements in the crown-apex direction, respecting the distance not to exceed the real working length. Always use a disposable, flexible fiber optic with a fine tip, around 0.3 mm diameter, to better diffuse the red laser inside the root canals;

» At the end, irrigate the root canals abundantly with the irrigating solution, aspirate, dry, use the intracanal calcium hydroxide medication (for at least 14 days), and perform the coronal sealing;

» In the second session, perform root canal obturation and coronal sealing; and

» If deemed necessary, a second session of photodynamic therapy (before obturation of the root canals) can be performed, following the same Protocol described above.

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