

Photodynamic Disinfection of Dental Impressions as a New Competitive Method to the Conventional Cleansing Procedures

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Abstract: The aim of the present study was to evaluate the efficiency of Photodynamic method as a disinfection procedure to the Prosthetic Dentistry. The conventional cleansing procedures were applied in comparison. Second generation photosensitizers from the group of phthalocyanines were synthesized and applied for photodynamic studies of inactivation of pathogenic microorganisms associated with dental impressions. The results showed that Photodynamic disinfection (PDD) can be successfully applied for the samples of dental impressions contaminated experimentally and especially the samples of human's origins. PDD as a new procedure was more efficient and competitive to the conventional disinfections of impressions.

Keywords: Dental impressions, photodynamic therapy, disinfection.

INTRODUCTION

Photodynamic therapy (PDT) is a new and promising alternative method for inactivation of pathogenic microorganisms. Antimicrobial PDT appears as very actual treatment modality in Dentistry due to anatomical features of oral cavity [1-4]. The method is based on the photochemical action employing three components: a photoactive dye (photosensitizer, PS) activated by proper light in the presence of oxygen [5]. The mechanisms of photosensitization direct the generation of reactive oxygen species (ROSs) [6]. The generation of singlet oxygen *via* energy transfer from the triplet excited state of PS to the molecular oxygen is defined as Type II mechanism of photosensitization. The parallel mechanism goes *via* an electron transfer from the triplet excited state of PS to surrounding molecules (Type I) [7]. Extremely toxic to the cells oxygen forms like free radicals and mainly singlet oxygen are formed [8]; the oxidative stress becomes very high and leads to cell death [6]. Photodynamic therapy characterizes as local treatment modality, because the photosensitizer accumulates selectively to the pathogenic than to surrounded normal cells [7].

Presently, PDT is still under development as therapeutic approach for clinical treatment of local infections of oral cavity, especially for that caused from multiresistant pathogens [7]. The clinical application of

PDT in Dental medicine is approved for Europe with a "FotoSan" (phenothiazine dye) and since 2010 this photosensitizer is commercially available. The application of photodynamic procedure for disinfection of dental impressions and prevention of cross-contamination between patient, dentist and dental technician is still novel field for common Dental practice [9].

The aim of the present study was to evaluate efficacy of the photodynamic disinfection as a method for cleansing of contaminated with pathogenic microorganisms' dental impressions under experimental and natural conditions.

MATERIALS AND METHODS

Experimental Groups

Standard disk specimens (7 mm in diameter and 4 mm thickness), made of different impression materials: alginate (Cavex Color Change, Cavex); silicone – condensation type (Swiss Tec, Light body, Coltene); silicone – addition type (Cavex Silicon A injection, Light body, Cavex) and polyether (Impregum F, 3M ESPE), were used for *in vitro* investigations. The specimens were autoclaved (121 °C, 1 bar, 1 hour) and contaminated with three different test microorganisms: methicillin-resistant *Staphylococcus aureus* 1337 (MRSA), *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* (Robin) Berkhout 1923. Three photosensitizers (water-soluble metal phthalocyanines) were used for photodynamic disinfection – GaPc1, ZnPc1 and SiPc. PS were applied and activated by LED lamp in the spectral range 635 nm (red light) at room temperature (RT).

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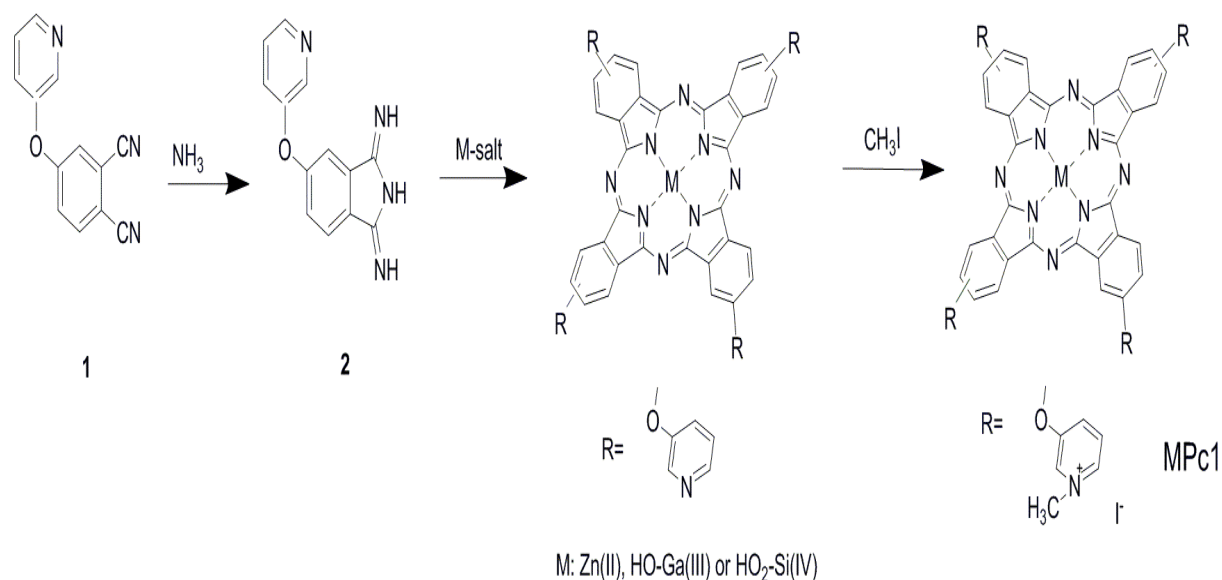


Figure 1: Chemical synthesis of the used phthalocyanines.

Forty eight dental impressions made of the same impression materials (12 of each type) were used for *in vivo* investigations. They were from different patients and four species of microorganisms were identified from them: *Str. viridans*; *Staphylococcus* - coagulasa negative; non – pathogenic *Neisseria* and *Pneumococcus*. Four photosensitizers were used for PDD: GaPc1, ZnPc1, SiPc and the commercially available FotoSan (CMS Dental), based on tylenol. Source of light was LED lamp in the spectral range 635 nm (red light) at RT. The chemical structure of the used phthalocyanines is shown on Figure 1.

Experimental Samples

Generally thirty experiments were carried out. The specimens were distributed into sterile microbiological plates (Tissue culture test plate, 12 wells – PS, TPP, Switzerland) and were sunk into microbial suspension 0, 5 McFarland in physiological solution for 5 minutes. Every row of specimens in the plate was incubated with different test microorganism. After that one specimen of each well was moved into another plate. Three different plates were completed and the specimens were sunk into photosensitizers (GaPc1, ZnPc1 and SiPc – solutions into 0,9 % NaCl, 10 µl/ml) in 10 min. After that the specimens were moved into different plates and were irradiated with red light 635 nm in 10 min. of each side. They were used for making cultures. One specimen of each well was utilized for making control culture without disinfection. Control cultures were made from the test microorganism suspensions also. The results were under review after 24 hours.

Humans Originated Samples

Material for making culture was taken from each impression with sterile tampon before disinfection. Each impression was sunk into PS (GaPc1, ZnPc1, SiPc and FotoSan) in 10 min and after that irradiated with red light 635 nm in 10 min. Material with sterile tampon was taken after disinfection also and the results were under review after 24 hours.

Statistical Significance Test Methods

Alternative analysis and parametrical test with U – criterion were used in the investigation ($p = 0,05$).

RESULTS

Experimental Impressions Study

Photodynamic disinfection (PDD) of dental impression materials with PS GaPc1 showed 40-90 % effectiveness only for the alginate samples. The specimens made of the other three groups of materials were all sterile. PDD with PS ZnPc1 and SiPc had different percent (%) of effectiveness for the samples made of different materials. Results of *in vitro* experiments are shown in the following figures (Figures 2, 3 and 4):

Results of Natural Impressions Study

The following microorganisms were identified from 12 alginate impressions: *Str. viridans* – in all the impressions; *Staphylococcus* - coagulasa negative – in

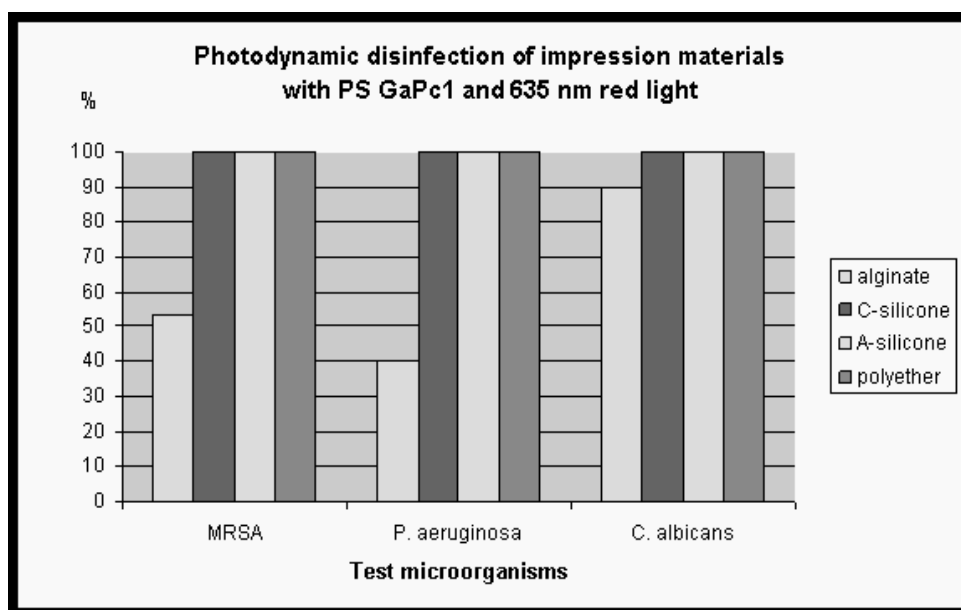


Figure 2: Photodynamic disinfection of impression materials (PS GaPc1, red light 635 nm).

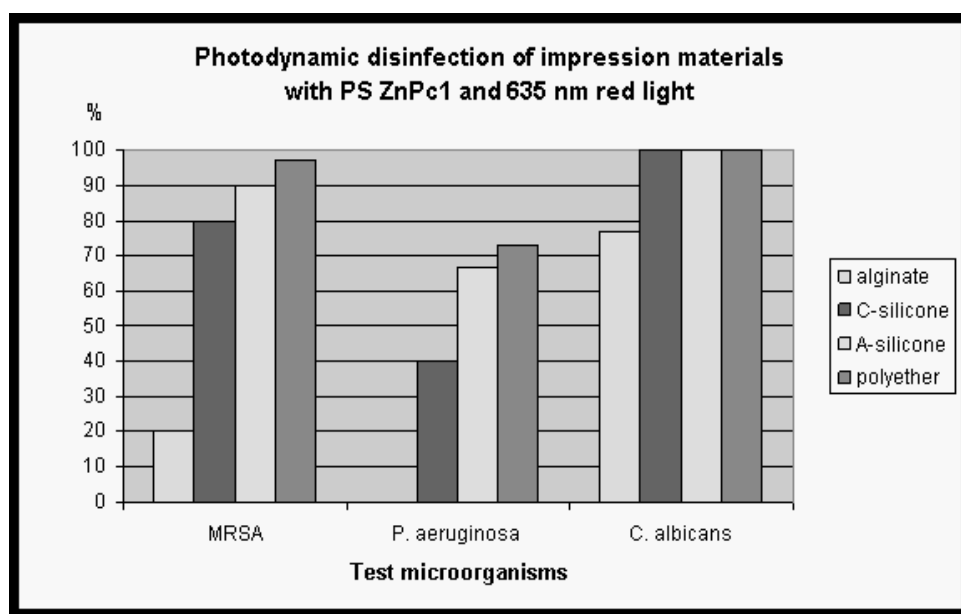


Figure 3: Photodynamic disinfection of impression materials (PS ZnPc1, red light 635 nm).

*The effectiveness of disinfection of the alginate samples contaminated with *P. aeruginosa* is 0 %.

8 of them; non – pathogenic *Neisseria* – in 9 and *Pneumococcus* – in 7 impressions. The microorganisms in 12 silicone - type C impressions were: *Str. viridans* – in all of them; *Staphylococcus* - *coagulasa* negative – in 4; non – pathogenic *Neisseria* – in 4 and *Pneumococcus* – in 4 impressions. From 12 silicone – type A impressions were isolated: *Str. viridans* – in 9; *Staphylococcus* - *coagulasa* negative – in 9; non – pathogenic *Neisseria* – in 8 and *Pneumococcus* – in 6 impressions. The microorganisms identified from 12 polyether impressions were: *Str. viridans* – in all of them;

Staphylococcus - *coagulasa* negative – in 6; non – pathogenic *Neisseria* – in 9 and *Pneumococcus* – in 6 impressions.

All the impressions were sterile after the photodynamic disinfection with all four PS: GaPc1, ZnPc1, SiPc and FotoSan (100 % effectiveness).

Statistical Findings

Statistical analysis formulated the following Zero hypothesis (H0): There is no difference in the

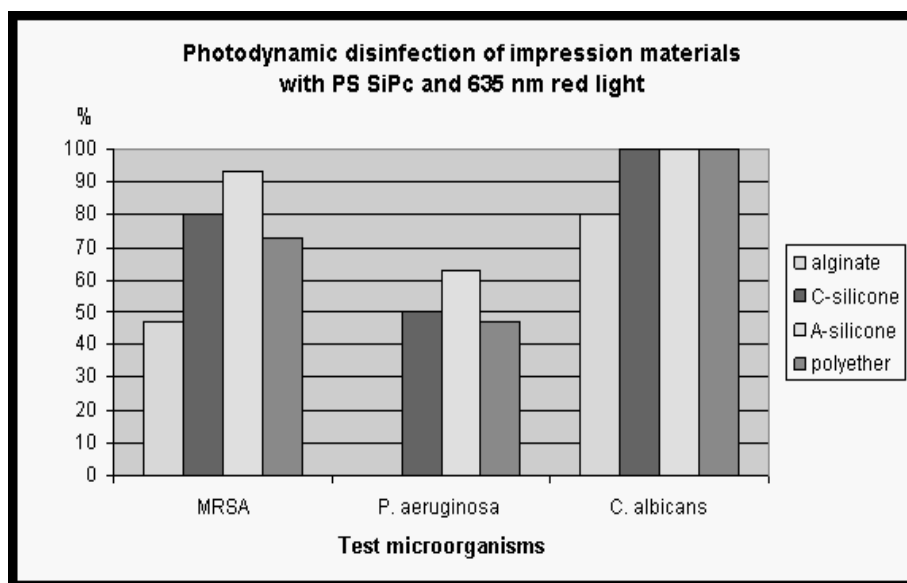


Figure 4: Photodynamic disinfection of impression materials (PS SiPc, red light 635 nm).

*0 % effectiveness of disinfection of the alginate samples contaminated with *P. aeruginosa*.

effectiveness of the investigated disinfectants. If there is difference, it is accidental and statistical insignificant. Parametrical test denied H_0 and showed that disinfectants are factor for decreasing number of microorganisms.

DISCUSSION

Impression materials and in particular irreversible hydrocolloid, also known as alginate, are some of the most commonly used dental materials. Alginate impressions are used to generate gypsum casts used for numerous applications, including treatment planning for restorative and orthodontic care, and fabricating removable prostheses. As with any hydrocolloid, alginates are approximately 85% water and are prone to distortion caused by expansion associated with imbibition (absorption of moisture) or shrinkage due to moisture loss [10]. In addition to water evaporation, impression shrinkage is related to syneresis and associated water exudation onto the impression surface caused by continuing contraction of the colloidal skeletal network even in 100% humidity [11]. Consequently, alginate impressions are not dimensionally stable, leading to decreased dimensional accuracy over time [10, 11]. Alginate impression materials are easy to use, less expensive, with quick setting time. The setting time can be controlled with the temperature of water used. They are mildly flavored. But they have a lot of disadvantages that include less accurate reproduction of details as compared with elastomeric impression materials, poor dimensional stability, and that they are messy to work with [11].

Photodynamic disinfection of alginate showed lower effectiveness than the other impression materials in reference to different photosensitizers. The reason was in the worse physical properties, the big porosity and the lower Shore hardness [11]. This facilitated the penetration of the microorganisms in depth. The red light used for irradiation also was not able to reach deep layers of the material.

The lower effectiveness of PS ZnPc1 (Figure 3) and SiPc (Figure 4) than GaPc1 (Figure 2) was due to different chemical structure and properties of the photosensitizers. The aggregation behavior of metal phthalocyanines depends on the concentration, on the metal ion into ligand, on the kind of substituent and on the polarity of solvent, and temperature [12]. The photodynamic sensitizers for applications in PDT are presumably the water soluble compounds or the compounds able to formulate into a functional carrier system in order to be acceptable in water surrounding. The properties of the excited states of PS are responsible for the photodynamic activity. The triplet state is mainly affected by the central metal ion in the ligand. The triplet quantum yield is known to increase for the larger atoms due to the heavy atom effect. Metallophthalocyanines (MPc) are easily aggregating in solution, which influences the photophysical characteristics negatively and leads to a lowering of the photodynamic effect [12].

External layer of the cytoplasm membrane of Gram (+) bacteria facilitates the penetration of the PS in the cell. Grams (+) bacteria external cover includes plasma

membrane, periplasmic space and peptidoglycan. Grams (-) bacteria have additional outer membrane, built by lipopolysaccharide and protein (Konopka and Goslinski, 2007) [3]. This is the reason for the fact that the Gram-positive bacteria are sensitive to the photodynamic action of photosensitizers independently on their charge and hydrophobicity, whereas the Gram-negative bacteria and fungi are resistant to anionic and neutral phthalocyanines [7, 13]. *P. aeruginosa* is exceptionally resistant Gram (-) bacterium [14, 15] and grows even into disinfectants and this fact explained the lowest level of disinfection regarding to this microorganism.

Microbe number of the bacteria in the oral cavity is lower then this in the suspensions of the test microorganisms (0, 5 McFarland = 10^8 colony forming units/ml (cfu/ml)). This fact can explain 100 % effectiveness of the photodynamic disinfection in clinical conditions in reference to different photosensitizers.

Every impression is soiled with blood, saliva, gingival fluid, dental plaque. All body fluids are contaminated with opportunist and pathogenic bacteria. These facts give an opportunity the impression to become a source of cross-infection.

CONCLUSION

This study suggests new alternative method for disinfection of impression materials by using photodynamic therapy as prevention of cross-contamination between patient, dentist and dental technician.

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