most common tool of dentists to treat oral infections, may correlate with antibiotic resistance.

Antibiotics have helped man to cope with bacterial infections to date, but the pathogens have become resistant to most of the antibiotics and the difficulty to develop new antibiotics threatens to return mankind to the pre-antibiotic era.

New antimicrobial strategies are therefore needed for example in dentistry in order to avoid excessive usage of antibiotics for treatment of periodontal, endodontic or mucosal topical infections caused by bacterial or yeast biofilms.

Summary

It is an aim of the present disclosure to provide methods of antimicrobial treatment while decreasing the risk of developing resistance in pathogens.

In particular, it is an aim to provide an approach for treating biological surfaces primarily by using light.

The present disclosure is based on the idea of treating biological surfaces with a combination of high and low energy photons simultaneously. It has surprisingly been found that the simultaneous use of high and low energy photons on the target area of a surface will give a biocidal effect that is better than the use of such photons separately.

It would appear – although this is merely one possibility, and the scope of the present disclosure is not limited to the following explanation – that low energy photons will penetrate deep into the surface, such as tissue, causing a heating effect, whereas high energy photons will have a biocidal effect on the surface. Thus, low energy photons upon absorption by the tissue will have a tissue heating effect of more than 1 °C, for example 1.5 to 3.5 °C, such as 2.7 °C, up to a depth of about 2 cm. In live (i.e. blood-filled) tissue, this would seem to increase the oxygen partial pressure and blood circulation that subsequently stimulates the metabolism of the cells and potentially promotes immune reactions. High energy photons, for example having an energy level of twice that of the low energy

photons, have an endogenous bacteria killing effect although the depth to which the photons penetrate into the tissue is smaller than for the low energy photos.

Thus, the same target area can be activated through photon up-conversion reactions where two or more photons are simultaneously absorbed and cause the target molecule(s) to excite to a higher energy state.

In one aspect, the present disclosure provides a non-therapeutic method of treating biological surfaces with electromagnetic radiation in the form of light of two different energy levels, a first light with photons having a majority energy in the range from 3.5 eV to 2.8 eV and a second light with photons having a majority energy in the range from 1.24 eV to 2.48 eV, wherein photons of the first light and the second light are simultaneously directed against the biological surface.

In another aspect, the present disclosure provides the use of a sensitizer in the manufacture of a medicament for the topical treatment of an infection on a mammal, wherein said sensitizer is to be applied to a part of skin or mucous membrane and said part is to be subsequently or simultaneously subjected to first photons with a majority energy between 2.8 eV and 3.5 eV; and second photons with a majority energy between 1.24 eV and 1.65 eV.

In yet another aspect, the present disclosure provides the use of an antibacterial factor in the manufacture of a medicament for use in combinatorial therapy for treating oral biofilm conditions of a mammal that is characterised by cavity formation and parodontitis; wherein said medicament is to be administered simultaneously, separately, or sequentially with a photosensitizer, wherein the antibacterial factor comprises at least one of the following: antibacterial blue light, disinfectant, reactive oxygen generators, and oxygen transporters.

In one example, a method is provided wherein the target area is contacted with a photosensitizer and then the target area thus treated will be subjected to the combination of first photons having a majority energy between 1.24 eV and 1.65 eV and second photons having a majority energy between 2.8 eV and 3.5 eV, said first and said second photons making up a majority, preferably more than 90 %, of all photons directed towards the target area.

A further example provides a photosensitizer for use in the topical treatment of an infection on a mammal, wherein said sensitizer is applied to a part of skin or mucous membrane and that part is subsequently or simultaneously subjected to first photons with a majority energy between 2.8 eV and 3.5 eV; and second photons with a majority energy between 1.24 eV and 1.65 eV.

In one example, a photosensitizer is provide for use in topical treatment of an infection on a mammal, wherein said sensitizer is first applied to a part of the skin or mucous membrane and that part is subsequently or simultaneously subjected to photons with a majority energy between 3.17 eV and 2.95 eV and 1.56 eV and 1.45 eV, respectively. Such energy levels correspond to wave lengths of about 390 to 420 nm and 795 to 855 nm, respectively.

A still further aspectprovides a kit for treatment of microbial, viral or fungal infections of tissue, in biofilm, saliva, skin, plaque, on teeth surfaces and in mucous membranes, comprising an optoelectronic component and device thereof capable of simultaneously emitting a first light consisting of high energy and a second light consisting of low energy photons, said first and said second light amounting to at least 80 % of all light emitted from the optoelectronic component or device, and at least one photosensitizer which can be activated by at least either of the high energy and low energy photons.

In yet another aspect, the present disclosure provides a kit comprising an optoelectronic device capable of emitting high energy photons with majority energy between 2.8 eV and 3.5 eV and low energy photons with majority energy between 1.24 eV and 1.65 eV, together with a photosensitizer or a plurality of photo-sensitizers, capable of carrying out a method for achieving a sustained antimicrobial effect in preventive and curative dental/oral care for long term use.

In yet another aspect, the present disclosure provides a kit comprising an optoelectronic device, in particular of the kind disclosed herein, capable of emitting two wavelengths simultaneously or at a time interval of 0.001 to 1000 ms, for example about 100 ms, from each other, optionally together with a photosensitizer or a plurality of photosensitizers,

capable of carrying out a method for achieving a sustained antimicrobial effect in preventive and curative dental/oral care for long term use.

In yet another aspect, the present disclosure provides a kit comprising an optoelectronic device having a light emitting component that has two or more light emitting surfaces (EPIs), optionally together with a photosensitizer or a plurality of photosensitizers, capable of carrying out a method for achieving a sustained antimicrobial effect in preventive and curative dental/oral care for long term use.

More specifically, the present disclosure is characterized by what is stated in the independent claims.

Considerable advantages are obtained. The use of high and low energy photons to target endo- and exogenous molecules gives rise to a target molecule site specific treatment due to low life-time of reactive or high energized oxygen.

According to the disclosure the high energy photons are being absorbed by endogenous (intracellular) molecules to generate reactive oxygen singlets and reactive oxygen. Simultaneously low energy photons are being absorbed exogenously (extracellular) by the photo-sensitizer resulting in reactive oxygen singlets and reactive oxygen. The reactive oxygen singlets and reactive oxygen species will inactivate, kill and otherwise reduce micro-organisms such as bacteria, virus and fungus in tissue, biofilm, saliva, skin, plaque and teeth surface.

The treatment will achieve good tissue penetration. It makes it possible to give antibacterial treatment to different areas of pathogen at the same time as two or more different energy photons can target molecules in different areas. Different energy photons have also different tissue therapeutic and tissue stimulating effects. The combined high and low energy photons can affect bacterial communication as they might have deleterious effect in bacteriophages, which contain genetic material or other molecules. The light may also have effects in the production, formation or activating of such communicating molecules assessed as quorum sensing.

It appears that the high energy photons are typically being absorbed by species relating to or involved with the intracellular oxidative stress responses. They are therefore capable of disrupting pathogen treatment adaptation. One example of such a species is the flavin group of the peroxidase enzyme.

The high and low energy photons can be used with several different kinds of photosensitizers, wherein the activation can take place through different mechanisms such as heat generation, oxygen radicals and singlet oxygen. By utilizing treatment combination where pathogens are targeted with two or more unspecific yet fundamentally different mechanisms, efficient antibacterial treatment can be achieved. In one example, ICG with low energy photons is used with high energy photons to give photo hyperthermia therapy (ICG acts 80 % through heat generation and 20–15 % through singlet oxygen formation) to pathogen membranes. High energy photons can be used for activating endogenous porphyrin molecules inherent in bacteria. Such molecules have high quantum yields and act mainly through singlet oxygen, resulting in localized oxidative bursts.

One important benefit of the combination of endogenous antibacterial therapy, in which the photons target inherent bacteria molecules, with exogenous photodynamic or photothermal therapy is that it can solve the issue that added exogenous photosensitizers tend to bleach out from target area during treatment.

However, endogenous antibacterial light therapy is not limited to the presence of an exogenous photosensitizer.

Targeting the endogenous molecules inherent in bacteria with photons gives an effect which is independent of the photosensitizer attachment and uptake. This helps to balance the treatment so that areas with less photosensitizer will have as good treatment as the areas with more photosensitizer. The photo bleaching effect of endogenous antibacterial therapy to pathogen endogenous molecules has also antipathogenic function as these molecules are essential for the pathogen unlike the added exogenous photosensitizer.

In long term, the targeting of bacteria endo- and exogenously gives the best effect *in vivo* against many bacteria as effectivity of exogenous treatment is limited to photosensitizer attachment and/or intake to target pathogen.

For example, simultaneously absorbing 1.53 eV and 3.06 eV photons can excite endogenous porphyrins creating antibacterial effect in addition to tissue healing effect. The high energy photons reduce the formation of biofilm extracellular polysaccharides matrix which gives synergies with exogenous PDT and reduces pathogenicity of biofilms.

Different photosensitizer, photon energy and treatment parameters can be used to target different age biofilms in different part of its life cycle. The composition of, for example dental plaque and biofilms varies from individual to individual. People with low incidence of caries show different bacterial amounts, different species and different phylogenetic diversity within the dental plaque, when compared to the high incidence of caries especially in the early days of plaque formation.

With the present disclosure people can be efficiently treated, irrespective of whether they are of high or low caries incidence.

The present treatment can also be used for treating other than biological materials having biological surfaces. Examples include equipment and parts of equipment covered by biofilms. Biofilms, also referred to as biofouling, can be found generally in industrial water systems, in the medical and process industries, including the paper and pulp industry, as well as in the food industry.

Next examples will be examined in more detail with reference to the attached drawings.

Brief Description of the Drawings

Figure 1 is a photograph showing dye plaque specificity as observed in room light with hamamatsu 1394 and NIR light source;

Figure 2 is a diagrammatic depiction of gray level fluctuation to indicate dye light absorption;

Figure 3 is a bar chart showing the antimicrobial effect of chlorhexidine potentiated with dual wavelength PDT according to an embodiment of the invention;

Figure 4 is a bar chart showing the antimicrobial effect of PDT treatment on 1, 2 and 4 days old *Streptococcus mutans* biofilms;

Figure 5 is a bar chart showing the efficiency of double wavelength and single wavelength treatments on 4 days old biofilms;

Figure 6 is a chart showing the antibacterial effect of light having a wave length of 405 nm compared to PDT; and

Figure 7 is a bar chart showing the antimicrobial effect of PDT treatment after 14 days on *Streptococcus mutans* biofilms

Claims

1. A non-therapeutic method of treating biological surfaces with electromagnetic radiation in the form of light of two different energy levels, a first light with photons having a majority energy in the range from 3.5 eV to 2.8 eV and a second light with photons having a majority energy in the range from 1.24 eV to 2.48 eV, wherein photons of the first light and the second light are simultaneously directed against the biological surface.

2. The method according to claim 1, wherein

- non-coherent radiant light energy is generated at at least two different energy levels, a first and a second energy level;
- from the non-coherent radiant light energy there is provided first light having a wavelength corresponding to the majority energy of the first energy level, and second light having a wavelength corresponding to the majority energy of the second energy level; and
- the first and second light are then simultaneously directed against the biological surface.

3. The method according to claim 1 or 2, wherein the biological material of the surface is subjected to endogenous and exogenous excitement, in particular so as to generate reactive oxygen singlets or reactive oxygen species or both.

4. The method according to any of the preceding claims, comprising preventing or combatting biological contamination of surfaces, such as microbial or viral or fungal contamination of biological tissues.

5. The method according to any of the preceding claims, comprising cosmetic treatment or antimicrobial or antiviral or antifungal therapy.

6. The method according to any of the preceding claims, wherein the light is used as such.

7. The method according to any of claims 1 to 6, wherein the light is combined with at least one photo-sensitive substance for treating the surface using photodynamic therapy.

8. The method according to any of the preceding claims, wherein the high energy photons and low energy photons are applied in conjugation with at least one exogeneous photo-sensitizer, which can be activated with the low energy photons.

9. The method according to any of the preceding claims, wherein a photosensitizer is provided for use in topical treatment of mammal tissues, wherein said sensitizer is to be applied to a superficial part of the tissue, such as on mammal skin or on a mucous membrane and the part thus treated is to be subsequently or simultaneously subjected to light at two different wavelengths.

10. The method according to any of the preceding claims, wherein the light consists of a first light having high energy photons with a majority energy between 2.8 eV and 3.5 eV; and a second light having low energy photons with a majority energy between 1.24 eV and 1.65 eV.

11. The method according to any of the preceding claims, wherein a photo-sensitizer is to be contacted with micro-organisms, such as bacteria, virus and fungus, in a target, such as tissue, in biofilm, saliva, skin, plaque and on teeth surfaces or mucous membranes, and wherein the photo-sensitizer is to be applied on the target in the form of an aqueous solution, an alcohol containing solution, a hydrophilic gel, a hydrophobic gel, a hydrophilic polymer, a hydrophobic polymer or a paste, lotion, tape, tablet, plaster or band-aid.

12. The method according to any of the preceding claims, wherein the biological surface is selected from tissues, biofilms, saliva, skin, plaque and teeth surfaces and mucous membranes.

13. The method according to any of the preceding claims, wherein high energy photons absorbed by endogenous molecules and low energy photons absorbed by exogenous molecules are simultaneously emitted from an optoelectronic component or device.

14. The method according to any of the preceding claims, comprising emitting high energy and low energy photons using feed-in voltage or current that is alternated or pulsed at 1Hz to 1 GHz frequency independently from each other.

15. The method according to any of the preceding claims, comprising using an optoelectronic device which includes an optional detector, such as a photo detector, to detect photo luminescence of the endogenous and exogenous molecules or their photo decomposition side products.

16. The method according to any of the preceding claims, comprising using an optoelectronic device having a plurality of semiconductor chips that are connected in series or in parallel, the chips exhibiting emission energy that can be varied in the range of 2.48eV and 1.24 eV and in the range of 3.5eV and 2.8 eV, respectively.

17. The method according to any of the preceding claims, comprising using during a first period of time a first photosensitizer and during a second period of time a second photosensitizer, which is different from the first photosensitizer.

18. The method according to claim 16 or 17, wherein the first photosensitizer and the second photosensitizer are activated using first light and second light, respectively.

19. The method according to any of claims 16 to 18, wherein the first photosensitizers are used in combination, or alternatingly, or at least one of them is used at a predetermined point of time during the treatment.

20. The method according to any of the preceding claims, wherein the photosensitizer(s) is(are) selected from the group of natural compounds comprising hypericin, curcumin, phenalenone derivatives, Cercosporin, psoralen, xanthotoxin, Angelicin, alpha-Terthienyl, Phenylthepatriyne, THC, Cannabidiol (CBD) and combinations thereof, and the group of synthetic photosensitizers comprising RB (Rose Bengal), MB, Porphyrin derivatives, Curcumin derivatives, Methylene Blue, Indocyanine Green, Erythosine, Phenalenone derivatives, Fullerene derivatives, Xanthene derivates and combinations thereof, or at least one photosensitizer is selected from the group of natural compounds listed above, and at least one photosensitizer is selected from the group of synthetic compounds listed above.

21. The method according to any of the preceding claims, wherein light energy, in particular non-coherent radiant light energy, is directed towards the biological surface at a first wavelength from 400 to 430 nm, preferably at a dosage of 1 to 120 J/cm^2, and in

particular with a power density of from about 10 to about 2500 mW/cm^2 for a period of time from 0.5 s to 120 min, and at a second wavelength from 780 to 830 nm, preferably at a dosage of 1 to 120 J/cm^2, and in particular with a power density of from about 10 to about 2500 mW/cm^2 for a period of time from 0.5 s to 120 min.

22. The method according to any of the preceding claims, wherein the light is produced by one or several light-emitting diodes.

23. Use of a sensitizer in the manufacture of a medicament for the topical treatment of an infection on a mammal, wherein said sensitizer is to be applied to a part of skin or mucous membrane and said part is to be subsequently or simultaneously subjected to first photons with a majority energy between 2.8 eV and 3.5 eV; and second photons with a majority energy between 1.24 eV and 1.65 eV.

24. The use according to claim 21, wherein the infection is a microbial, viral or fungal infection of tissue, in biofilm, saliva, skin, plaque, on teeth surfaces and mucous membrane.

25. The use according to claim 22 or 23, comprising at least one photosensitizer selected from the group of natural compounds comprising hypericin, curcumin, phenalenone derivatives, Cercosporin, psoralen, xanthotoxin, Angelicin, alpha-Terthienyl, Phenylthepatriyne, THC, Cannabidiol (CBD) and combinations thereof, and the group of synthetic photosensitizers comprising RB (Rose Bengal), MB, Porphyrin derivatives, Curcumin derivatives, Methylene Blue, Indocyanine Green, Erythosine, Phenalenone derivatives, Fullerene derivatives, Xanthene derivates, resveratrol, berry extracts, such as lingonberry and blueberry including polyphenolic compounds and/or anthocyanine compounds and combinations thereof, or the photosensitizer being the combination of at least one photosensitizer is selected from the group of natural compounds listed above and at least one photosensitizer is selected from the group of synthetic compounds listed above.

26. Use of an antibacterial factor in the manufacture of a medicament for use in combinatorial therapy for treating oral biofilm conditions of a mammal that is characterised by cavity formation and parodontitis; wherein said medicament is to be administered simultaneously, separately, or sequentially with a photosensitizer, wherein

the antibacterial factor comprises at least one of the following: antibacterial blue light, disinfectant, reactive oxygen generators, and oxygen transporters.

27. The use according to any of claims 23 to 26, wherein the photosensitizer is subjected to light energy, in particular non-coherent radiant light energy, at a first wavelength from 400 to 430 nm, preferably at a dosage of 1 to 120 J/cm^2, and in particular with a power density of from about 10 to about 2500 mW/cm^2 for a period of time from 0.5 s to 120 min, and at a second wavelength from 780 to 830 nm, preferably at a dosage of 1 to 120 J/cm^2, and in particular with a power density of from about 10 to about 2500 mW/cm^2 for a period of time from 0.5 s to 120 min.

28. A kit for treatment of microbial, viral or fungal infections of tissue, in biofilm, saliva, skin, plaque, on teeth surfaces and in mucous membranes, comprising an optoelectronic device capable of simultaneously emitting a first light consisting of high energy and a second light consisting of low energy photons, said first and said second light amounting to at least 80 % of all light emitted from the optoelectronic component or device, and at least one photosensitizer which can be activated by at least either of the high energy and low energy photons.

29. A kit comprising an optoelectronic device capable of emitting high energy photons with majority energy between 2.8 eV and 3.5 eV and low energy photons with majority energy between 1.24 eV and 1.65 eV, together with a photosensitizer or a plurality of photo-sensitizers, capable of carrying out a method for achieving a sustained antimicrobial effect in preventive and curative dental/oral care for long term use.

30. A kit comprising an optoelectronic device, in particular of the kind disclosed in claim 29 or 30, capable of emitting two wavelengths simultaneously or at a time interval of 0.001 to 1000 ms, for example about 100 ms, from each other, optionally together with a photosensitizer or a plurality of photosensitizers, capable of carrying out a method for achieving a sustained antimicrobial effect in preventive and curative dental/oral care for long term use.

31. A kit comprising an optoelectronic device having a light emitting component that has two or more light emitting surfaces (EPIs), optionally together with a photosensitizer or a

plurality of photosensitizers, capable of carrying out a method for achieving a sustained antimicrobial effect in preventive and curative dental/oral care for long term use.

32. A kit according to any of claims 28 to 31, further comprising a sensor capable of monitoring treatment progression, said sensor in particular being capable of detecting treatment progression and of producing a regulation signal based on the progress of the treatment, said sensor being coupled to the optoelectronic device do adjust the light emitted from the optoelectronic device depending on the regulation signal.

33. A kit according to any of claims 28 to 32, comprising an optoelectronic device exhibiting the shape of a tooth brush, or the shape of a mouth piece which can be inserted in a mouth between the biting surfaces of the teeth, or the shape of a rod like illuminator.

34. The kit according to any of claims 28 to 33, wherein the optoelectronic device used comprises micro-spectrometer sensors, temperature sensors, light sensors, pH sensors, force sensors, gyroscopes, pressure sensors or combinations thereof.

35. The kit according to any of claims 28 to 34, comprising a tooth whitening substance capable of being used with a dual wavelength light source or together with a photosensitizer.

36. The kit according to any of claims 28 to 35, comprising a photosensitizer in form of water soluble effervescent tablet and further comprising a hand held light applicator capable of emitting dual light photons.

39. The kit according to any of claims 28 to 36, comprising a photosensitizer in the form of a water soluble effervescent tablet, gel, or paste, and further comprising a one-time use mouth piece and light applicator.

38. The kit according to any of claims 28 to 37, comprising a photosensitizer in the form of photosensitizers incorporated or inherently present in berry extracted mouth rinse.

39. The kit according to any of claims 28 to 38, comprising hydrogen peroxide as a potentiating compound

40. The kit according to any of claims 28 to 39, wherein the optoelectronic device is capable of emitting light, in particular non-coherent light, at a first wavelength from 400 to 430 nm, preferably at a dosage of 1 to 120 J/cm^2, and in particular with a power density of from about 10 to about 2500 mW/cm^2 for a period of time from 0.5 s to 120 min, and at a second wavelength from 780 to 830 nm, preferably at a dosage of 1 to 120 J/cm^2, and in particular with a power density of from about 10 to about 2500 mW/cm^2 for a period of time from 0.5 s to 120 min.

41. The kit according to any of claims 28 to 39, wherein the optoelectronic device comprising light-emitting diode(s) as a light source.