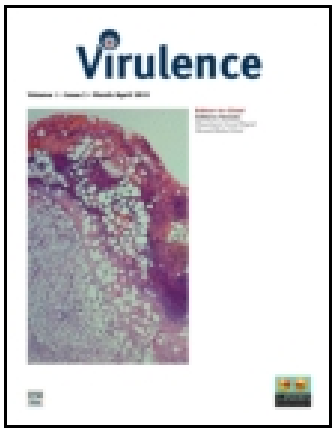

Virulence
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kvir20

Non-conventional therapeutics for oral infections

Robert P Allaker[a] & CW Ian Douglas[b]

[a] Oral Microbiology; Barts and The London School of Medicine & Dentistry; Queen Mary University of London; London, UK

[b] Oral Pathology; School of Clinical Dentistry; University of Sheffield; Sheffield, UK

Accepted author version posted online: 10 Feb 2015.

Virulence 6:3, 196–207; April 2015; Published with license by Taylor & Francis Group, LLC

Non-conventional therapeutics for oral infections

Robert P Allaker[1,*] and CW Ian Douglas[2]

[1]Oral Microbiology; Barts and The London School of Medicine & Dentistry; Queen Mary University of London; London, UK; [2]Oral Pathology; School of Clinical Dentistry; University of Sheffield; Sheffield, UK

Keywords: antimicrobial peptides, caries, detergents, enzymes, light-activation, nanoparticles, periodontitis, probiotics

As our knowledge of host-microbial interactions within the oral cavity increases, future treatments are likely to be more targeted. For example, efforts to target a single species or key virulence factors that they produce, while maintaining the natural balance of the resident oral microbiota that acts to modulate the host immune response would be an advantage. Targeted approaches may be directed at the black-pigmented anaerobes, *Porphyromonas gingivalis* and *Prevotella intermedia*, associated with periodontitis. Such pigments provide an opportunity for targeted phototherapy with high-intensity monochromatic light. Functional inhibition approaches, including the use of enzyme inhibitors, are also being explored to control periodontitis. More general disruption of dental plaque through the use of enzymes and detergents, alone and in combination, shows much promise. The use of probiotics and prebiotics to improve gastrointestinal health has now led to an interest in using these approaches to control oral disease. More recently the potential of antimicrobial peptides and nanotechnology, through the application of nanoparticles with biocidal, anti-adhesive and delivery capabilities, has been explored. The aim of this review is to consider the current status as regards non-conventional treatment approaches for oral infections with particular emphasis on the plaque-related diseases.

Introduction

The oral cavity provides habitats for a wide diversity of microorganisms including bacteria, yeasts and viruses; members of all groups being associated with oral infections. Bacteria are the predominant components of this resident microflora, and the diversity of species found in the oral cavity reflects the wide range of endogenously derived nutrients, the varied types of habitat for colonization including surfaces on the teeth, mucosa and tongue, and the opportunity to survive as a biofilm.[1,2] However, the relationship between this microflora and the host can be disrupted in a number of ways, resulting in the development of disease of the oral structures. These are mainly localized and include dental caries (dental decay), gingivitis, periodontitis (gum disease), candidiasis, endodontic (root canal) infections, orthodontic infections and peri-implantitis.[3]

Most bacterial infections within the oral cavity are polymicrobial in nature, and it is quite unusual to find any that are clearly due to a single species. The relative contribution of different bacterial components in such infections is thus difficult to determine. Oral infections may arise either from an endogenous source, i.e., one yielding microorganisms normally found in the mouth, such as the main plaque-related diseases of dental caries and periodontal disease, or from an exogenous source yielding microorganisms not normally found as part of the oral microflora (e.g. syphilis, tuberculosis). Plaque-related diseases are probably the most common bacterial diseases occurring in man. Dental caries is a destructive condition of the dental hard tissues that, if unchecked, can progress to inflammation and death of vital pulp tissue, with eventual spread of infection to the periapical area of the tooth and beyond. The disease process involves acidogenic plaque bacteria, including *Streptococcus mutans*, *Streptococcus sobrinus, Lactobacillus* spp., *Actinomyces* spp and *Bifidobacterium* spp.,[3,4] whereas periodontal diseases can involve both the soft and hard tissues and are the most common inflammatory destructive conditions that affect man. They are initiated by components of the plaque that develops on the hard root surface adjacent to the soft tissues of the supporting periodontium and may be confined to the gingiva (gingivitis) or extend to the deeper supporting structures with destruction of the periodontal ligament and the alveolar bone that supports the teeth (periodontitis). Such loss of attachment, with associated periodontal pocket formation, may ultimately lead to loosening and loss of the affected teeth. *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* are regarded as the major pathogens in advancing periodontitis.[5] Furthermore it has been recently suggested that there is an association between the oral microbiota and systemic disease, such as cardiovascular disease and complications during pregnancy.[6,7]

The prevention of dental caries and the periodontal diseases is traditionally targeted at the mechanical or non-specific control of dental plaque as this is the precipitating factor. This is carried out to maintain plaque at levels compatible with health and so prevent the breakdown of microbial homeostasis which increases disease risk. However, the individual response of the host and other confounding factors can influence disease initiation and progression. Antimicrobial and antiplaque compounds in oral care products represent a valuable complement to mechanical plaque control. Such strategies should ideally prevent plaque

*Correspondence to: Robert P Allaker; Email: R.P.Allaker@qmul.ac.uk
Submitted: 08/29/2014; Revised: 10/30/2014; Accepted: 10/31/2014
http://dx.doi.org/10.4161/21505594.2014.983783

biofilm formation without affecting the biological equilibrium within the oral cavity, which is inhabited by up to 1000 different species of bacteria at 10^8–10^9 bacteria per mL saliva or mg dental plaque.[8] However, with ever increasing antibiotic resistance and a public desire for more 'natural' therapies, there is an increased need to minimise antibiotic use and develop novel treatments for oral diseases that do not involve conventional antimicrobial agents.

Dental Plaque Disruption

Enzymes

A number of oral streptococci, including *Streptococcus mutans*, produce a range of water-soluble and insoluble glucan extracellular polysaccharides *in vivo*.[9] The different types of glucans are synthesized by different glucosyltransferases, particularly α-(1–>3)-linked glucans (synthesized by GTF B) and α-(1–>6)- and α-(1–>3)-linked glucans (synthesized by GTF C).[9] The water insoluble types (α-1→3) are thought to contribute to the cariogenicity of the plaque at a site through its physical retentive nature, its ability to provide a large diffusion volume for dietary sugars and as a substrate for further adhesion of bacteria. Consequently a desirable goal would be to distrupt this feature of the plaque biofilm with glucanase enzymes (dextranase and mutanse). Both dextranases and mutanases are able to suppress the accumulation of dental plaque in human volunteers, in animals or *in vitro* depending upon the experimental system employed.[10-12] Such enzymes would be applied directly to dental plaque where GTFs are still active, which raises the question of their likely longer term efficacy. However, these enzymes have been found, to influence glucan synthesis by GTFs themselves in terms of linkage remodelling and branching and this appears to have an impact on the formation, maturation and physical properties of the glucans present.[13] Consequently there is reason to believe that such enzymes could be a valuable addition to the control of the plaque biofilm.

Another approach to enzymic disruption of biofilms is the use of deoxyribose nucleases (DNAases). While it has been known since the 1940's that bacteria can release DNA extracellularly,[14] it is only relatively recently that biofilms have been found to contain a significant amount of intact extracellular DNA (eDNA). This is present either because of cell death or active secretion, or both, and there is now strong evidence that this DNA contributes to the matrix of the biofilm, an important structural component of its scaffold.[15] It seems logical then that application of exogenous DNAases could degrade this scaffold and so provide a means of disrupting the integrity of the biofilm and/or its growth. Indeed, a number of workers recently have shown that DNAase treatment of *in vitro* biofilms can weaken the structure and result in release of bacteria.[16-18] However, DNAases are relatively expensive to produce currently and for application on a large clinical scale improvements in production methods will be required. Nonetheless, enzymic disruption of the plaque biofilm does look a promising way forward for the future.

Detergents

An alternative to enzymic disruption of the biofilm matrix is chemical disruption and a number of ionic detergents can bind to bacteria and their matrix, which could be effective biofilm disrupting agents. While cationic detergents have been used in commercial products for some years, often referred to as pre-brushing mouth washes, there have been conflicting claims about their effectiveness. Despite the numerous claims and counterclaims, recent systematic reviews of the literature have failed to show clear advantages of detergent mouthwashes over good oral hygiene instruction on the removal of dental plaque.[19,20] However, it is possible that the failure of these products to realize their potential has been due to the mode of their delivery, since the tendency is for the agent to bind largely to the surface of the biofilm. One of the most commonly used agents in such pre-brushing mouthwashes is cetylpyridinium chloride and recent work has concentrated on exploring alternative delivery methods. These methods include incorporation of cetylpyridinium chloride within cholesterol liposomes[21] and in nanoemulsions.[22] Although not yet tested clinically, the nanoemulsions look particularly effective since *in vitro* testing has shown inhibitory effect on *S. mutans* biofilms[23] and using a multispecies biofilm grown in an artificial mouth system, showed good penetration into the biofilm. Furthermore, it was superior to chlorhexidine in reducing the depth of artificial carious lesions and in mineral loss in this system.[22] Perhaps combinations of nanoemulsions of detergents and enzymic disruption agents, to aid even more effective penetration, would provide optimum plaque disruption.

Light-activated Killing

The fact that bacteria can be killed by light in the presence of a sensitizing agent was discovered 100 years ago by Reitz in 1908[24] but it is only recently that the possibility of using this technology for the control of oral infection has been considered.[25] The process of killing microorganisms with light depends upon the generation of cytotoxic singlet oxygen and OH radicals (reactive oxygen species; ROS), which are formed by the excitation of a photactivateable agent or sensitizer. The result of excitation is that the sensitizer moves from its electronic ground state to a triplet state that then interacts with cell components to generate ROS.[26] One of the particular values of light-activated killing is that resistance to the action of singlet oxygen is less likely to become a major concern and spread widely, unlike that experienced with chemical antimicrobial agents. Despite the general truth of this, recent data have shown that spontaneous mutants that are resistant to photoactivated killing can arise.[27]

A sensitizer should bind avidly to the bacterial cell and/or be taken up by it, thus a number of sensitisers are highly charged molecules,[28] the most commonly tested of which have been tricyclic dyes, (e.g., methylene blue, erythrosine), phenothiazine dyes (e.g. toluidine blue O), tetrapyrroles (e.g., porphyrins) and furocoumarins (e.g. Psoralen). **Table 1** shows a number of the sensitisers that have been used in recent studies, some of which are

Table 1. Example photosensitisers used in recent studies

Sensitizer	Reference
Phenalen 1	29
Indocyanine green	30
Methylene blue	31,32
Toluidine blue ortho	33
Malachite green	34
Eosin-Y	35,36
Rose bengal	35,36
Curcumin	36
Nile blue derivatives	37
BIODIPY derivatives	38
Dicationic 5,15-diarylporphyrins	39
Haematoporphyrin monomethyl ether	40
Meso-substituted porphyrins	27
Radachlorin	41

tried and tested agents while others are newly synthesized variants of previously used categories of agents (e.g., porphyrins).

A sensitizer ideally should absorb light at red to near-infrared wavelengths because longer wavelengths tend to be more penetrative. However, red light sources with narrow wavelength bandwidths tend to be expensive, whereas LED light sources (blue or red) are commonly available in dental surgeries so finding sensitizing agents that are compatible with such light sources would be an advantage for use against oral infections. These dental LED lights are used to cure resin-based composite restorative materials. A dental light was used to assess killing of *S. mutans*, *A. actinomycetemcomitans* and *Enterococcus faecalis* by a commercial preparation of haematoporphyrin sensitizer (Photosan).[42] The Photosan dye was readily taken up by the Gram-positive species but not by *A. actinomycetemcomitans* unless 10% EDTA was used to aid entry of the dye through the outer membrane. Around 3 to 4 $\log_{10}$ reduction in viable count was achieved, which demonstrates the potential of these commonly used light sources for photoactivated killing. However, others have reported that dental LEDs are not as effective at killing *A. actinomycetemcomitans* as the more powerful indium-gallium-aluminum-phosphate lasers.[41] Inefficient killing of Gram-negative species relative to Gram-positive by photoactivation has been known for almost 30 years[43] as well as the value of adding a membrane-disrupting agent to enhance access of the photo-sensitizer. However, other effective approaches include using agents with strong cationic charge to enhance binding to Gram-negative bacteria[44] and liposome encapsulation to enhance delivery by fusion with the membrane.[45,46] In each case though, the degree of killing will still depend upon the efficiency of the photosensitizer being used.

The majority of work on light-activated killing has been performed using suspensions of planktonic bacteria; however a crucial requirement for application to many disease states, including the dental plaque-related diseases, is the ability to kill microorganisms growing as biofilms. In this phenotype, microorganisms are known to be much more resistant to killing by chemical agents, including antibiotics, and so assessment of efficacy should always include assays on biofilms. Over twenty years ago, Wilson et al[47] showed that oral bacteria could be killed by low power laser radiation in the presence of methylene blue both as planktonic suspensions and when growing as a biofilm on an agar surface. Whether these are true biofilms has been the subject of debate for some years, but more recently *in vitro* biofilm-grown *S. mutans* cells were killed by up to 3 $\log_{10}$ fold when treated with erythrosine and white light (500–650 nm).[48] Photoactivated killing has also been assessed against *E. faecalis* biofilms grown for 4 days and 4 weeks within the root canals of extracted teeth and while this did reduce bacterial viability, maximal improvement was gained when it was combined with chemomechanical treatment. Nonetheless, dental LEDs with common blue light-absorbing photosensitizer dyes can eradicate *E. faecalis* biofilm, although requiring increased sensitizer concentration over that required to kill planktonic bacteria.[36]

There are a number of other aspects that should be considered in relation to the therapeutic use of light-activated killing of biofilms on host surfaces: (1) direct toxicity of the sensitizer, (2) indirect toxicity of the sensitizer in terms of "by-stander" damage to adjacent host cells, (3) penetration into the biofilm, and (4) light exposure time required to kill bacteria within *in vivo* biofilms. Answers to all of these questions are not readily available or universally applicable to all bacteria. However, in relation to (1) and (2) it is known that sensitizing agents can give rise to phototoxic effects on keratinocytes and fibroblasts[49] and phototoxic reactions in experimental animal oral tissues have been recorded, although the effect appears to be short lived.[50] The photosensitizer erythrosine has an advantage over some other dyes because it is currently used in dentistry to visualize dental plaque in vivo, and so its lack of direct toxicity on the host is well established. For phototherapeutic use in periodontitis, the dye needs to be applied subgingivally prior to fiber-optic laser light activation. However, in disease the periodontal site has a marked flow of serum into the pocket (gingival crevicular fluid) and most photosensitizers lose a degree of activity in the presence of extraneous protein and some have virtually no effect in the presence of serum, blood or saliva. This is because the agents complex with proteins and host cells in the crevicular fluid which effectively competes for binding to bacteria. Despite these theoretical limitations, a commercial PDT system designed for use in periodontal patients (Periowave, Ondine Biopharma, Vancouver, Canada) has been shown to provide some clinical advantage when combined with conventional root surface debridement (RSD) compared to RSD alone.[51,52] However, ideally mono-therapy by PDT for periodontitis would be advantageous as PDT monotherapy has been shown to provide not only some improvement in clinical signs but also reduction of local proinflammatory cytokines and reduction in numbers of *P. gingivalis* and *A. actinomycetemcomitans*.[53] Nonetheless, the modest clinical improvements achieved so far might be enhanced if methods for better retention of the photosensitiser at the site could be found.

An approach that might achieve this is the use of nanoparticles to deliver the ROS-generating system. Such systems may have better sustainability in the subgingival environment by being taken up into the plaque biofilm.[54] Nanoparticles that contain a conventional sensitizer and have antibacterial action have been known for some time, for example the

polysiloxane polymers containing embedded methylene blue and gold nanoparticles described by Perni et al.[55] However, more recently similar systems have been developed that are independent of a chemical sensitizer. Chong et al.[56] produced nanoparticles of a boron-dipyrromethene polymer that is cationic and that on photoactivation with white light generates ROS at the particle surface.

Selective killing

Another approach for phototherapy is to take advantage of an 'intrinsic sensitizer' within the target bacteria, rather than relying on the vagaries of dye binding and penetration. Several studies have indicated the use of porphyrins as potential antimicrobial agents and these can interfere with bacterial heme uptake systems as well as generate ROS. Porphyrins are metal-chelating groups and the oral anaerobes belonging to the *Porphyromonas* and *Prevotella* genera include species that accumulate heme compounds on their cell surface in the form of a black pigment. Soukos et al[57] found that light in the wavelength range 380–520 nm rapidly and selectively killed oral black-pigmented bacteria in pure cultures. They hypothesized that the killing effect was due to photo-excitation of porphyrins on the surfaces of these bacteria, so making the effect quite specific. A similar phenomenon has been found with *A. actinomycetemcomitans* in which blue light without sensitizer reduced the viable count of a suspension by 5 $\log_{10}$, which appeared to be due to endogenous flavin-like or porphyrin-like compounds.[58] In contrast there was no effect of blue light on *Escherichia coli* because no singlet oxygen species was generated presumably because no similar endogenous photosensitisers were detected.[58] Exploiting natural porphyrins as photosensitisers certainly has value if it can be successfully applied to subgingival plaque in patients with periodontitis. Indeed, light applied to the oral surfaces of periodontitis patients shows a selective reduction of 4 black-pigmented species.[57]

Functional Inhibition

As summarised above and for obvious reasons, the approach most often taken for treating infection is to attempt to kill or eradicate the offending organisms. However, where the identity of the offending organism is less clear, for example in chronic infections associated with complex mixtures of organisms (viz. periodontitis), an alternative approach to therapy that maintains an element of homeostasis of the population would be beneficial. One approach, as explained later, is to replace the pathogenic strain with a non-pathogenic strain ('replacement therapy'). Another is to consider a disease process in terms of the "virulence burden" that the host experiences, regardless of the identity of the contributing organisms. Limiting the functionality of these critical virulence factors would allow the host's defense mechanisms to deal with the organisms present as a collection. The concept moves away, therefore, from targeting specific organisms or groups of organisms and toward targeting their products. Of course, a disadvantage of this approach is that knowledge of the principal virulence factors involved is a necessity.

Microbial proteases

One important class of bacterial virulence factors that are a potential target for such a therapeutic approach is extracellular proteases. Some of these have broad specificity, e.g. trypsin-like proteases,[59,60] while others are very specific (e.g., IgA protease, which cleaves the hinge region of the immunoglobulin molecule).[61] There are 4 main classes of proteases; (1) serine proteases, (e.g., trypsin-like, elastase), (2) cysteine proteases (e.g., gingipains), (3) Aspartic proteases, (e.g., *Candida albicans* Saps), and (4) metallo-proteases (e.g., microbial keratinases). Individually or collectively these classes of enzymes play critical roles in infection, both in terms of direct tissue destruction and more subtle effects, such as activation of host proteases,[62] inactivation of host protease inhibitors[63] and disruption of cytokine networks. Another important class of proteases in terms of microbial physiology is the caseinolytic proteases that are not extracellular. These are termed Clp proteases and they regulate protein quality and turnover including damaged proteins and transcriptional regulators.[64] Clp-dependent proteolysis has been implicated in expression of extracellular virulence factors, such as cytotoxins, and in resisting adverse conditions inside host cells. Loss of Clp functionality has been shown to render some pathogens more sensitive to innate host defenses, including host antimicrobial peptides.[65] Thus, bacterial proteases are a useful target for therapeutics not only to prevent their direct and indirect effects on host tissue and systems, but also because proteases fulfil a nutritive and defensive role for the bacteria.[66,67] **Table 2** shows a series of example target proteases of pathogenic bacteria, including certain oral organisms.

Protease inhibitors

Inhibitors of therapeutic value are usually thought of as inorganic/synthetic agents; however, they could also be "host products" appropriately manipulated. A known example of a host product is salivary histatin 5, which is an inhibitor of both host and bacterial proteases implicated in periodontal disease.[68] This antimicrobial (host defense) peptide also shows general inhibitory effect against a range of organisms but particularly fungi[69,70] and the domain responsible for this anti-protease activity resides in a 14 residue C-terminal sequence. The peptide is known to have an effect at mucosal surfaces that are readily accessible to saliva, but periodontal sites are not normally penetrated by salivary secretions. Thus, histatin would have to be used as a therapeutic agent directly applied subgingivally in a suitable vehicle.

The main classes of inorganic or synthetic inhibitors are chelators, oxidizing agents, thiol-blocking agents, heavy metal ions, methanethiosulfonates and organo-mercurials. Chelators, such as EDTA, are common inhibitors of metalloenzymes, while lanthanides inhibit Ca^{2+}-requiring proteases, because Ln^{3+} ions replace Ca^{2+} and form an inactive enzyme-substrate complex.[71,72] However, lanthanides also activate the conversion of trypsinogen to trypsin.[73] A recently discovered inhibitor of Clp proteases was identified by high throughput screening as N- (1- (2-aminoethyl) -lH-tetrazol- 5-yl) -3-chlorobenzamide and which is termed F2.[74] This inhibitor has pleiotropic effects on the bacterial cell

Table 2. Selected examples of bacterial proteases, their preferred cleavage sites and example inhibitors

Enzyme	Source	Cleavage site	Inhibitor
Serine and Cysteine proteases			
Glutamyl endopeptidase I	*Staphylococcus aureus*	Glu-Phe or Glu-Val	None
Exfoliative toxin A	*S. aureus*	Glu-X	
IgA1-specific protease	*Haemophilus influenzae Neisseria meningitidis*	Pro-Ser, Pro-Thr	None specific
C5a peptidase	*Streptococcus agalactiae Streptococcus pyogenes*	His-Lys	None specific
Trepolisin	*Treponema denticola*	Phe-X	None specific
Prolyl tripeptidyl-peptidase	*P. gingivalis*	X-Y-Pro-X	None specific
Omptin	*E. coli Yersinia pestis*	Arg-Arg, Arg-Lys	thiol-blocking agents
Clostripain C11	*Clostridium histolyticum*	Arg-X, Lys-X	oxidizing agents, thiol-blocking agents, heavy metal ions
Gingipain R			
Gingipain K	*P. gingivalis*	Arg-X Lys-X	thiol-blocking agents 1-(3-phenylpropionyl) piperidine-carboxylic acid-[4-amino-1(S)-(benzothiazole-2-carbonyl)butyl] amide,
Sortase	ubiquitous in Gram-positive bacteria	Leu-Pro-X-Thr-Gly	methanethiosulfonates or organo-mercurials
Clp peptidases	Ubiquitous	Met-Ala	ATP-dependent
Metalloproteinase			
Pseudolysin	*Pseudomonas aeruginosa*	Phe-X or Gly-Leu	EDTA, EGTA, phenanthroline
Vibrio collagenase	*Vibrio parahaemolyticus*	X-Gly	
Clostridium collagenases	*Clostridium perfringens*	X-Gly	sulfonylated amino acid hydroxamates
Aeruginolysin	*Ps. aeruginosa*	Leu-Gly or Gly-Gly	
Mirabilysin	*Proteus mirabilis*	Leu-Gly (in IgA)	
Fragilysin	*Bacteroides fragilis*	Leu-Gly Gly-Leu	EDTA, DTPA
Sialoglycoprotein endopeptidase	*H. influenzae*	Arg-Asp	

because the Clp protease regulates a wide range of genes[75] and also F2 has been shown to increase the effectiveness of bacterial killing by human whole blood, indicating that this compound can augment innate immune defenses.[74] Consequently the therapeutic effect of F2 may be enhanced at tissue sites where high levels of antimicrobial peptides are produced e.g periodontal sites.

An important role for Clp proteases in the virulence of *P. gingivalis* has been identified. First, ClpC and ClpXP expression is elevated in biofilms and the ClpC and XP proteases are necessary for internalization into epithelial cells. ClpB is required for intracellular survival.[76] Consequently, inhibitors of the Clp proteases, such as F2, have potential applications in the treatment of periodontitis.

P. gingivalis gingipain proteases

The gingipain proteases of the periodontal pathogen *P. gingivalis* are a particularly interesting target in relation to periodontitis. There are 2 major gingipains, arginine-specific gingipain (RgpA & B) and a lysine specific gingipain (Kgp). Despite the fact that the Rgps are in approximately a 3-fold excess over Kgp,[77] the latter appears to be the most important enzyme for virulence and for nutrient assimilation. The specific mechanism (s) by which this enzyme is involved in these functions is not absolutely clear but a *kgp* mutant has been shown to be unable to accumulate haemin at the cell surface (loss of black pigmentation) which results in reduced oxygen tolerance and reduced virulence factor expression.[78,79] In addition, the inability to sequester iron may lead to the down regulation of other traits required for maximal expression of virulence *in vivo*. Consequently, Kgp appears to be a useful target enzyme for direct inhibition and work by Curtis et al.[80] developed and tested an inhibitor specific for Kgp (1- (3-phenylpropionyl) piperidine-3 (R, S)—carboxylic acid—[4-amino-1 (S)-(benzothiazole-2-carbonyl) butyl] amide (A71561)). This agent showed no inhibition of the Rgps, and did not inhibit growth of *P. gingivalis* on complex media; however, pretreatment of *P. gingivalis* with this agent prior to infection in a murine model significantly reduced pathogenicity. In contrast, pretreatment of *P. gingivalis* with an Rgp inhibitor, leupeptin, did not affect virulence.

Despite this, it is known that the RgP gingipains act in concert with Kgp gingipain to contribute to the entire virulence of the organism, particularly through disruption of the host complement system.[81] Therefore, a single agent that inhibits both gingipain proteases has clinical therapeutic potential. Kataoka et al[82] recently developed such a dual inhibitor through structure-based drug design. It has potent antibacterial activity against *P. gingivalis in vitro* and reduced gingival inflammation in a beagle dog periodontitis model.

Probiotics and Prebiotics

Probiotics

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Probiotics have been used successfully to control gastro-intestinal diseases and appear to act through colonisation resistance and / or immune modulation. Likewise, studies also suggest that probiotics have the potential to modify the oral microbiota. However these may only be successful over the short term. Experimental studies and clinical trials have demonstrated that certain gastrointestinal bacteria, including *Lactobacillus* and *Bifidobacterium* spp,

may also control the growth of oral microorganisms, including cariogenic streptococci associated with disease.

Mechanisms of probiotic action within the oral cavity can possibly be suggested from gastrointestinal studies[83] whereby the introduction of microorganisms as a therapeutic tool for the prevention and treatment of dental caries and periodontal disease could possibly act as follows within the oral environment.

1. Direct interactions within dental plaque. These could possibly include the disruption of plaque biofilm formation through competition for binding sites on host tissues and other bacteria, and competition for nutrients. The production of antimicrobial compounds that inhibit oral bacteria may also be a significant mechanism. It is known that lactic acid bacteria produce a range of antimicrobial agents including organic acids, hydrogen peroxide, low molecular weight antimicrobial peptides, bacteriocins and adhesion inhibitors.[83]
2. Indirect probiotic actions within the oral cavity, including the modulation of aspects of both innate and specific immune function. Within this context, it is possible that lactic acid bacteria can interact with immunocompetent cells, such as macrophages and T-cells, leading to cytokine production and subsequent effects on overall immunity.[83]

Lactobacillus rhamnosus CG,[84] *L. casei,*[85] *L. reuteri*[86] and *Bifidobacterium* DN-173 010[87] have all demonstrated the potential to alter colonisation of cariogenic bacteria and thus prevent dental caries. The oral administration of probiotics has also been explored in the control of periodontal disease. Krasse et al.[88] demonstrated a reduction in plaque levels and gingival inflammation with the application of *L. reuteri* to subjects with moderate to severe gingivitis. To achieve optimal effects, simultaneous use of multiple species may be required as described using *S. oralis*, *S. uberis* and a lactic acid-deficient variant of *S. rattus*, whereby the probiotic mouthwash was able to markedly affect the levels of cariogenic bacteria in saliva and periodontal pathogens in subgingival plaque.[89] However, although available data indicates an effect of probiotics on the oral microbiota, a more limited effect on clinical periodontal outcome measures is observed. There is a need for clinical trials where probiotics are used as adjuncts to standard periodontal treatment.

'Replacement therapy' based upon biotechnological approaches has also been investigated. Techniques include gene inactivation to remove metabolites that may harm the host and the incorporation of genes to encode for antimicrobial compounds, for example bacteriocins that inhibit the growth of strains of the same species. Genetically modified bacteria, for example *S. mutans,* are being considered for replacement therapy in the control of dental caries. Using recombinant DNA methodology, a strain of *S. mutans* was made lactate dehydrogenase deficient by the deletion of virtually all of the genetic sequence encoding this activity. To then compensate for the resulting metabolic imbalance, an alcohol dehydrogenase gene from *Zymomonas mobilis* was introduced. No detectable lactic acid during growth was produced from the resulting clone. This strain was also significantly less cariogenic than the parent strain in gnotobiotic- and conventional-rodent models of disease. In addition it was found to colonise the teeth of conventional rats to the same extent as the parent strain using both aggressive-displacement and preemptive-colonisation approaches. The clone was shown to be genetically stable and did not revert to producing acid in both *in vivo* and *in vitro* test systems.[90]

Prebiotics

Prebiotic substances, non-digestible oligosaccharides and other selectively fermented food ingredients have been used to improve gastrointestinal health and are now receiving interest in relation to oral applications. The main mechanism by which prebiotics act is assumed to be via facilitating proliferation of commensal bacteria, with resulting probiotic effects.[91] Some prebiotics may also exert effects on the host mucosal immune and inflammatory systems, independent of their effects on commensal bacteria.[91] Examples of prebiotics include inulin-type fructans, maltodextrin, fructooligosaccharides and galactooligosaccharides. It is also known that the oral microflora of the child is influenced to a large extent by diet. Within this context it is of interest that human milk contains oligosaccharides that have prebiotic characteristics.[92]

Antimicrobial Peptides

Antimicrobial (host defense) peptides (AMPs) are a diverse group of molecules and include defensins, cathelicidins, histatins, neuropeptides, peptide hormones, and many other proven and putative peptides. These peptides are produced by many tissues and cell types; with phagocytic and epithelial cells as the predominant source. Peptide concentrations from these cells increase significantly following infection or injury. Human saliva and gingival crevicular fluid contains at least 45 individual antimicrobial proteins and peptides that can be classified into different functional classes[93] (**Table 3**). The majority of AMPs fall within the cationic peptide group and are generally defined as being 12-50 amino acids in length, with a net positive charge of +2 to +7 and up to 50% hydrophobic amino acids. This provides for an amphipathic design, consisting of spatially separated hydrophobic and charged regions, and permits intercalation of the peptide with microbial membranes. Direct antimicrobial activity against microorganisms has been considered, until recently, their primary function. However, many peptides may have no direct inhibitory activity at their physiological concentrations found within oral fluids. Conversely, AMPs may have an antimicrobial effect at the epithelial surface of the gingivae or at the secretion site from neutrophils where the local concentration could be higher than that found in oral fluids.

There is now increasing evidence that AMPs are multifunctional molecules of fundamental importance in host defense, modulating between aspects of the innate and adaptive immune systems. Recently, it has become apparent that AMPs stimulate a wide range of effects relevant to inflammation, innate immunity and adaptive immunity. This includes effects on innate immune cells, including neutrophils and epithelial cells, and in those cells

Table 3. Functional classes of antimicrobial peptides and proteins (with examples) found in the oral cavity

Cationic peptides	Bacterial agglutination and adhesion	Metal ion chelators	Peroxidases	Protease inhibitors	Activity against bacterial cell walls
Adrenomedullin	β-2-microglobulin	Calgranulins A & B	Lactoperoxidase	Cystatins	Lysozyme
β defensins 1-3	Fibronectin	Lactoferrin	Myeloperoxidase	Secretory leukoprotease inhibitor protein	Peptidoglycan recognition proteins
Cathelicidin (LL-37)	Proline-rich proteins	Psoriasin			
Histatins 1 and 3		Transferrin			
Neutrophil (α) defensins 1-4					

that bridge the innate and adaptive immune systems, including monocytes, macrophages and other antigen-presenting cells. These peptides have been shown to modify cellular functions such as chemotaxis, apoptosis, gene transcription and cytokine production. In addition, they have been shown to have roles in the stimulation of wound healing and angiogenesis.[94] The use of AMPs to control oral infections including periodontitis may therefore depend upon direct antibacterial, anti-inflammatory and/or immune modulatory actions.

Functional and structural diversity of AMPs and other innate defense molecules may well be necessary to help protect the oral epithelia from infection and maintain the balance of commensals and opportunistic pathogens required for health. Furthermore, AMP expression is partly regulated by the oral microbiota,[95] with the commensal bacteria often inducing peptide expression above that demonstrated with the more pathogenic species. Differences in AMP expression between periodontitis patients and healthy subjects has been identified using proteomic analyses.[96] Understanding the role of individual AMPs in oral disease may lead to the development of disease biomarkers and new therapies. AMPs also differ markedly in their antimicrobial activity against different oral species.[97] For example, the antimicrobial effects of adrenomedullin can vary considerably, with both fully resistant and sensitive (minimum inhibitory concentrations down to < 0.1 μg/ml) oral anaerobic species / strains.[98] When such MIC values are compared to concentrations of AM found in the gingival crevicular fluid (GCF) of patients with periodontal disease (approx. 1-2 μg/ml),[99] it is clear that adrenomedullin has the potential to influence the growth of the oral microflora *in vivo*.

Systems are being developed to target AMPs against given oral species using a targeting peptide, linker region and antimicrobial peptide component.[100,101] Other approaches include the modification of natural peptides to generate peptides with more favorable efficacy/toxicity profiles.[102] Alternatively, peptide mimetics have been designed and synthesized which retain the biological activity of an AMP but are advantageous as regards production costs, possess favorable therapeutic index and show stability under physiological conditions.[103] For example, mimetics based upon the defensin structure have demonstrated a high therapeutic index in pre-clinical studies.[104] With regards to the oral cavity, the development of novel antimicrobials should allow control of pathogens without loss of beneficial commensals.[105] Approaches that stimulate or restore the normal expression patterns of AMPs, rather than being used as exogenous therapeutic agents, may be particularly useful in the prevention of periodontal disease. For example, through the use of receptor activation or through the use of protease inhibitors to improve the longevity of AMPs or related receptors.[106] As with other agents, a combination of AMP use and mechanical debridement is likely to be most successful in the control of periodontitis.

Nanotechnology

Nanotechnology represents the ability to image, manipulate and model functionalities on the nanometer scale.[107] This discipline includes the use of nanoparticles which can be classified as particles of a size less than 100 nm. Properties of nanoparticles, for example, their active surface area, chemical reactivity and biological activity, are often far removed from those of a greater size. These characteristics should allow them to closely interact with microbial surfaces, and thus elicit an antimicrobial effect that is not solely due to released components. Metallic and other nanoparticles are now being combined with polymers and other base materials, and coated onto surfaces to ultimately provide a variety of potential applications within the oral cavity. The use of nanotechnology offers the possibility to control the formation of oral biofilms through the application of nanoparticles with biocidal, anti-adhesive and delivery capabilities.[108]

Nanoparticle based implant coatings should also offer both osteoconductive and antimicrobial functionalities to prevent dental implant failure. Such implant systems are increasingly being used to replace missing teeth, and most integrate with bone without complications. The condition peri-implantitis is a major cause of dental implant failure whereby the induced inflammatory changes in the soft tissues surrounding the implant lead to a progressive destruction of the supporting bone.[3] Current forms of treatment are often inadequate, with chronic infection often requiring implant removal and expensive resective and regenerative procedures in an attempt to restore and reshape the supporting tissue.[109]

Antimicrobial nanoparticles and control of oral biofilms

Metals have been used for centuries as antimicrobial agents. Silver, copper, gold, titanium and zinc have attracted particular attention, each having different properties and spectra of activity. Indeed, many oral products, including toothpastes, now incorporate powdered (micron-sized) zinc citrate or acetate to control

the formation of dental plaque.[110] With respect to nanoparticulate metals, the antimicrobial properties of silver[111] and copper[112] have received the most attention. Both of these have been coated onto or incorporated into various test materials, including the denture material Poly (methyl methacrylate) (PMMA).[113]

Nanoparticulate metal oxides have been of particular interest as antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal morphologies that have a high number of edges, corners and other potentially reactive sites.[114] On the other hand, certain metal oxides are now coming under close scrutiny because of their potential toxic effects to eukaryotic cells.[115] Oxides under consideration as antimicrobial agents include those of copper, zinc, titanium and tungsten. Studies have shown that some nanoparticulate metal oxides, such as ZnO, have a degree of selective toxicity to bacteria with a minimal effect on human cells at the concentrations employed.[116] Bacteria are far less likely to acquire resistance to metal nanoparticles than they are to other conventional and narrow-spectrum antibiotics. This is thought to occur because metals may act on a broad range of microbial targets, and many mutations would have to occur in order for the microorganisms to resist their antimicrobial activity.[117]

Quaternary ammonium poly (ethylene imine) (QA-PEI) antimicrobial nanoparticles have also been developed and incorporated into dental composite resins.[118] This approach may be particularly beneficial when compared to the currently used composite resins for hard tissue restoration, which are known to possess several disadvantages including development of biofilms on both teeth and the restorative material.

Anti-adhesive nanoparticles and oral biofilm control

Particles of a nano and micro size based upon the element silicon have been designed to rapidly deliver antimicrobial and anti-adhesive capabilities to the desired site within the oral cavity.[119] Companies use silica (often classed as 'micro fine', but with a particle size within the definition of nanoparticles) in toothpastes, and some have actively sought new directions in this area through the use of porous silicon and nanocrystalline silicon technology to carry and deliver antimicrobials, for example, triclosan. Other systems based upon silica have been investigated with respect to the control of oral biofilms. The use of nitric oxide-releasing silica nanoparticles to eradicate biofilm growth has been described. Bioactive glasses of the SiO_2-Na_2O-CaO-P_2O_5 system have been shown to possess antimicrobial activity through the release of ionic alkaline species over time.[120] Those in the form of amorphous nanoparticles, with a size range of 20 to 60 nm, may show an advantage over micron-sized material as the decrease in glass particle size should increase the active exchange surface of glass and surrounding liquid. This should then substantially increase ionic release into suspension and enhance antimicrobial efficacy. Chitosan, a biopolymer derived by the deacetylation of chitin occurring in the exoskeleton of crustaceans is positively charged and soluble in acidic to neutral solution, enabling it to bind to mucosal surfaces. Both chitosan nano- and microparticles have been investigated as a potential platform for local delivery of drugs within the oral cavity.[121]

The application of nano-scaled hydroxyapatite (nHA) particles has been shown to impact on oral biofilm formation and can also provide a re-mineralization capability. Biomimetic approaches based upon HA nanocrystals which resemble the structure at the nano-scale of abraded dental enamel crystallites, in theory should allow adsorbed particles to interact with bacterial adhesins, reduce bacterial adherence and hence impact on biofilm formation.[122] A number of oral health care products, including toothpastes and mouth rinses, have been developed containing nano-sized apatite particles with and without protein-based additives.[123,124] It is suggested that the efficacy of these compounds can be attributed to the size-specific effects of the apatite nanoparticulates. Casein phosphopeptide (CPP)—amorphous calcium phosphate (ACP) nanocomplex (Recaldent™/MI Paste™) is a particular technology based upon ACP and stabilized by casein phosphopeptide (CPP).[125] Use of this technology has demonstrated anticariogenic activity under both *in vitro* and *in vivo* test conditions. With reference to dental implants, numerous companies market synthetic HA materials as the 'optimal' osteoconductive implant coating available and some companies have developed nano-scaled varieties. Furthermore, combined nHA and nano zinc oxide (nZnO) coatings have shown much potential as regards antimicrobial activity and biocompatibility.[116]

Biocompatibility of nanoparticles within the oral cavity

Although the development and application of nanotechnology is of considerable interest, knowledge regarding the possible toxicity of nanotechnology products to humans is limited. In order to fully understand the mechanism of toxicity, a thorough knowledge of the toxico-kinetic properties of nanoparticles is required. Toxicology and biodynamic studies do suggest that silica, silicon, and chitosan nanoparticles are relatively safe if introduced *via* the oral route.[126] The safe use of nanotechnology and the design of nanomaterials for biological applications involve a thorough understanding of the interface between these materials and biological systems.[127] The nanoparticle characteristics of most importance as regards interaction with biological systems, whether mammalian or microbial, are chemical composition, surface function, shape and number of sides, porosity and surface crystallinity, size heterogeneity, roughness, and hydrophobicity or hydrophilicity.[128] In order to help prevent aggregation of nanoparticles, stabilizing (capping) agents that bind to the entire nanoparticle surface can be used; these include water-soluble polymers, oligo- and poly-saccharides, sodium dodecyl sulfate, polyethylene glycol and glycolipids.[129] An understanding of the interface between biological systems and nanomaterials should enable design features to be used to control the exposure, bioavailability and biocatalytic activities. A number of possible approaches are starting to be identified including changing the ability to aggregate, application of surface coatings, and altering charge density and oxidative state. However this may well compromise the intended selective toxicity of antimicrobial nanoparticles. It remains to be determined how potential mammalian toxicity issues will fully impact on the use of nanotechnology in the control of oral infections.

Conclusions

Further studies, including investigations into the cost effectiveness, specificity, safety aspects and patient acceptance of using non-conventional approaches in the control of oral infections will be required. Such strategies should ideally reduce plaque levels without affecting the overall biological equilibrium within the oral cavity. Selective light-activated killing, functional inhibition of specific virulence factors and microbial replacement therapy offer a more targeted approach, whereas the use of plaque disrupting agents, antimicrobial peptides and nanoparticles are relatively more general. Approaches that offer complementary modes of action will increase antimicrobial effectiveness when used in combination, particularly when improved delivery systems are employed. The increasing consumer demand for effective and safe oral care products will help to further drive the need to investigate non-conventional therapeutics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Marsh PD, Martin MV. Oral Microbiology, 5th Edition. London: Churchill Livingstone 2009.
2. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J Ind Microbiol 1995; 15:169-75; PMID:8519474; http://dx.doi.org/10.1007/BF01569822
3. Allaker RP, Hardie JM. Oral infections. (Chapter 20). In: Topley and Wilson's Microbiology and Microbial Infections (9th Edition). Arnold, London, 1998:3; 373-90.
4. Mantzourani M, Gilbert SC, Sulong HN, Sheehy EC, Tank S, Fenlon M, Beighton D. The isolation of bifidobacteria from occlusal carious lesions in children and adults. Caries Res 2009; 43:308-13; PMID:19494490; http://dx.doi.org/10.1159/000222659
5. Slots J, Bragd L, Wikstrom M, Dahlen G. The occurrence of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius* in destructive periodontal disease in adults. J Clin Periodontol 1986; 13:570-7; PMID:3462204; http://dx.doi.org/10.1111/j.1600-051X.1986.tb00849.x
6. Beck JD, Offenbacher S. Systemic effects of periodontitis: epidemiology of periodontal disease and cardiovascular disease. J Periodontol 2005; 76:2089-100; PMID:16277581; http://dx.doi.org/10.1902/jop.2005.76.11-S.2089
7. Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S. Periodontal disease and adverse pregnancy outcomes: a systematic review. Brit J Obstet Gynaecol 2006; 113:135-43; http://dx.doi.org/10.1111/j.1471-0528.2005.00827.x
8. Rosan B, Lamont RJ. Dental plaque formation. Microbes Infect 2000; 2:1599-607; PMID:11113379; http://dx.doi.org/10.1016/S1286-4579(00)01316-2
9. Banas JA, VickermanMM. Glucan-binding proteins of the oral streptococci. Crit Rev Oral Biol Med 2003; 14:89-99; PMID:12764072; http://dx.doi.org/10.1177/154411130301400203
10. Hamada S, Ooshima T, Masuda N, Sobue S. Effect of Dextranase prepared from *Spicaria violacea* on dental caries in the hamster. J Dent Res 1976; 55:552; PMID:1063768; http://dx.doi.org/10.1177/00220345760550034101
11. Inoue M, Yakushiji T, Mizuno J, Yamamoto Y, Tanii S. Inhibition of dental plaque formation by mouthwash containing an endo-alpha-1, 3 glucanase. Clin Prev Dent 1990; 12:1-14.
12. Jiao YL, Wang SJ, Lv MS, Jiao BH, Li WJ, Fang YW, Liu S. Characterization of a marine-derived dextranase and its application to the prevention of dental caries. J Ind Microbiol Biotechnol 2014; 41:17-26; PMID:24197466; http://dx.doi.org/10.1007/s10295-013-1369-0
13. Hayacibara MF, Koo H, Vacca-Smith AM, Kopec LK, Scott-Anne K, Cury JA, Bowen WH. The influence of mutanase and dextranase on the production and structure of glucans synthesized by streptococcal glucosyltransferases. Carbohydr Res 2004; 339:2127-37; PMID:15280057; http://dx.doi.org/10.1016/j.carres.2004.05.031
14. Avery OT, MacLeod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from Pneumococcus type III. J Exp Med 1944; 79:137-58; PMID:19871359; http://dx.doi.org/10.1084/jem.79.2.137
15. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. Science 2002; 295(5559):1487; PMID:11859186; http://dx.doi.org/10.1126/science.295.5559.1487
16. Hall-Stoodley L, Nistico L, Sambanthamoorthy K, Dice B, Nguyen D, Mershon WJ, Johnson C, Hu FZ, Stoodley P, Ehrlich GD, et al. Characterization of biofilm matrix, degradation by DNase treatment and evidence of capsule downregulation in *Streptococcus pneumoniae* clinical isolates. BMC Microbiol 2008; 8:173-89; PMID:18842140; http://dx.doi.org/10.1186/1471-2180-8-173
17. Nijland R, Hall MJ, Burgess JG. Dispersal of biofilms by secreted, matrix degrading, bacterial DNase. PLoS One 2010; 5:e15668; PMID:21179489; http://dx.doi.org/10.1371/journal.pone.0015668
18. Kaplan JB, LoVetri K, Cardona ST, Madhyastha S, Sadovskaya I, Jabbouri S, Izano EA. Recombinant human DNase I decreases biofilm and increases antimicrobial susceptibility in staphylococci. J Antibiot (Tokyo) 2012; 65:73-7; PMID:22167157; http://dx.doi.org/10.1038/ja.2011.113
19. Van Leeuwen MPC, Slot DE, Van der Weijden GA. Essential oils compared to chlorhexidine with respect to plaque and parameters of gingival inflammation: a systematic review. J Periodontol 2011; 82:174-94; PMID:21043801; http://dx.doi.org/10.1902/jop.2010.100266
20. Gunsolley JC. Clinical efficacy of antimicrobial mouthrinses. J Dent 2010; 38 Suppl 1:S6-10; PMID:20621242; http://dx.doi.org/10.1016/S0300-5712(10)70004-X
21. Cottenye N, Cui ZK, Wilkinson KJ, Barbeau J, Lafleur M. Interactions between non-phospholipid liposomes containing cetylpyridinium chloride and biofilms of *Streptococcus mutans*: modulation of the adhesion and of the biodistribution. Biofouling 2013; 29:817-27; PMID:23826726; http://dx.doi.org/10.1080/08927014.2013.807505
22. Lee VA, Karthikeyan R, Rawls HR, Amaechi BT. Anti-cariogenic effect of a cetylpyridinium chloride-containing nanoemulsion. J Dent 2010; 38:742-49; PMID:20600554; http://dx.doi.org/10.1016/j.jdent.2010.06.001
23. Karthikeyan R, Amaechi BT, Rawls HR, Lee VA. Antimicrobial activity of nanoemulsion on cariogenic *Streptococcus mutans*. Arch Oral Biol 2011; 56:437-445; PMID:21112582; http://dx.doi.org/10.1016/j.archoralbio.2010.10.022
24. Reitz A. Untersuchungen mit photodynamischen stoffen (Photobiologischen sensibilisatoren). Centr Bakteriol Parasitenek 1908; 45:270-85.
25. Meisel P, Kocher T. Photodynamic therapy for periodontal diseases: state of the art. J Photochem Photobiol B 2005; 79:159-70; PMID:15878121; http://dx.doi.org/10.1016/j.jphotobiol.2004.11.023
26. MacRobert AJ, Bown SG, Phillips D. What are the ideal photoproperties for a sensitizer? In: Bock G, Harnett S, eds. Ciba Foundation Symposium. Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use. Chichester: John Wiley 1989, 146.
27. Burda WN, Fields KB, Gill JB, Burt R, Shepherd M, Zhang XP, Shaw LN. Neutral metallated and meso-substituted porphyrins as antimicrobial agents against gram-positive pathogens. Eur J Clin Microbiol Infect Dis 2012; 31:327-35; PMID:21667268; http://dx.doi.org/10.1007/s10096-011-1314-y
28. Alves E, Costa L, Carvalho CM, Tomé JP, Faustino MA, Neves MG, Tomé AC, Cavaleiro JA, Cunha A, Almeida A. Charge effect on the photoinactivation of Gram-negative and Gram-positive bacteria by cationic meso-substituted porphyrins. BMC Microbiol 2009; 9:70; PMID:19368706; http://dx.doi.org/10.1186/1471-2180-9-70
29. Späth A, Leibl C, Cieplik F, Lehner K, Regensburger J, Hiller KA, Bäumler W, Schmalz G, Maisch T. Improving photodynamic inactivation of bacteria in dentistry: highly effective and fast killing of oral key pathogens with novel tooth-colored type-II photosensitizers. J Med Chem 2014; 57:5157-68; http://dx.doi.org/10.1021/jm4019492
30. Boehm TK, Ciancio SG. Diode laser activated indocyanine green selectively kills bacteria. J Int Acad Periodontol 2011; 13:58-63; PMID:21913603
31. George S, Kishen A. Photophysical, photochemical, and photobiological characterization of methylene blue formulations for light-activated root canal disinfection. Biomed Opt 2007; 12:34029-38; http://dx.doi.org/10.1117/1.2745982
32. Peloi LS, Soares RR, Biondo CE, Souza VR, Hioka N, Kimura E. Photodynamic effect of light-emitting diode light on cell growth inhibition induced by methylene blue. J Biosci 2008; 33:231-37; PMID:18535357; http://dx.doi.org/10.1007/s12038-008-0040-9
33. Lima JP, Sampaio de Melo MA, Borges FM, Teixeira AH, Steiner-Oliveira C, Nobre Dos Santos M, Rodrigues LK, Zanin IC. Evaluation of the antimicrobial effect of photodynamic antimicrobial therapy in an in situ model of dentine caries. Eur J Oralm Sci 2009; 117:568-74; http://dx.doi.org/10.1111/j.1600-0722.2009.00662.x
34. Prates RA, Yamada AM Jr, Suzuki LC, Eiko Hashimoto MC, Cai S, Gouw-Soares S, Gomes L, Ribeiro MS. Bactericidal effect of malachite green and red laser on *Actinobacillus actinomycetemcomitans*. J Photochem Photobiol B 2007; 86:70-6; PMID:16979345; http://dx.doi.org/10.1016/j.jphotobiol.2006.07.010
35. Rolim JP, de-Melo MA, Guedes SF, Albuquerque-Filho FB, de Souza JR, Nogueira NA, Zanin IC, Rodrigues LK. The antimicrobial activity of photodynamic therapy against *Streptococcus mutans* using different photosensitizers. J Photochem Photobiol B 2012; 106:40-46; PMID:22070899; http://dx.doi.org/10.1016/j.jphotobiol.2011.10.001
36. Pileggi G, Wataha JC, Girard M, Grad I, Schrenzel J, Lange N, Bouillaguet S. Blue light-mediated inactivation of *Enterococcus faecalis* in vitro. Photodiagnosis

Photodyn Ther 2013; 10:134-40; PMID:23769279; http://dx.doi.org/10.1016/j.pdpdt.2012.11.002
37. Vecchio D, Bhayana B, Huang L, Carrasco E, Evans CL, Hamblin MR. Structure-function relationships of Nile blue (EtNBS) derivatives as antimicrobial photosensitizers. Eur J Med Chem 2014; 75:479-91; PMID:24561676; http://dx.doi.org/10.1016/j.ejmech.2014.01.064
38. Caruso E, Banfi S, Barbieri P, Leva B, Orlandi VT. Synthesis and antibacterial activity of novel cationic BODIPY photosensitizers. J Photochem Photobiol B 2012; 114:44-51; PMID:22682365; http://dx.doi.org/10.1016/j.jphotobiol.2012.05.007
39. Orlandi VT, Caruso E, Tettamanti G, Banfi S, Barbieri P. Photoinduced antibacterial activity of two dicationic 5,15-diarylporphyrins. J Photochem Photobiol B 2013; 127:123-32; PMID:24041850; http://dx.doi.org/10.1016/j.jphotobiol.2013.08.011
40. Sun Y, Xing D, Shen L, Sun M, Fang M, Bi L, Sui Y, Zhang Z, Cao W. Bactericidal effects of hematoporphyrin monomethyl ether-mediated photosensitization against pathogenic communities from supragingival plaque. Appl Microbiol Biotechnol 2013; 97:5079-87; PMID:23615742; http://dx.doi.org/10.1007/s00253-013-4903-0
41. Moslemi N, Soleiman-Zadeh AP, Bahador A, Rouzmeh N, Chinifourus N, Paknejad M, Fekrazad R. Inactivation of *Aggregatibacter actinomycetemcomitans* by two different modalities of photodynamic thereapy using Toluidine blue O or radachlorin as photosensitisers: an in vitro study. Lasers Med Sci 2014 [Epub ahead of print]; PMID:24981641
42. Maisch T1, Wagner J, Papastamou V, Nerl HJ, Hiller KA, Szeimies RM, Schmalz G. Combination of 10% EDTA, Photosan, and a blue light hand-held photopolymerizer to inactivate leading oral bacteria in dentistry in vitro. J Appl Microbiol 2009; 107:1569-78; PMID:19457024; http://dx.doi.org/10.1111/j.1365-2672.2009.04342.x
43. Ehrenberg B, Malik Z, Nitzan Y. Fluorescence spectral changes of hematoporphyrin derivative upon binding to lipid vesicles, *Staphylococcus aureus* and *Escherichia coli* cells. Photochem Photobiol 1985; 41:429-35; PMID:3160054; http://dx.doi.org/10.1111/j.1751-1097.1985.tb03508.x
44. Banfi S, Caruso E, Buccafurni L, Battini V, Zazzaron S, Barbieri P, Orlandi V. Antibacterial activity of tetraarylporphyrin photosensitizers: an in vitro study on Gram negative and Gram positive bacteria. J Photochem Photobiol B 2006; 85: 28-38; PMID:16737820; http://dx.doi.org/10.1016/j.jphotobiol.2006.04.003
45. Nisnevitch M, Nakonechny F, Nitzan Y. Various delivery systems for the sensitisers Photodynamic antimicrobial chemotherapy by liposome-encapsulated water-soluble photosensitizers. Bioorg Khim 2010; 36:396-402; PMID:20644595
46. Chen C, Chen C, Ysang J, Tsai T. Liposome-encapsulated photosensitizers against bacteria. Recent Pat Antiinfect Drug Discovery 2013; 8:100-7; http://dx.doi.org/10.2174/1574891X113089990011
47. Wilson M, Dobson J, Harvey W. Sensitization of oral bacteria to killing by low-power laser radiation. Curr Microbiol 1992; 25:77-81; PMID:1369193; http://dx.doi.org/10.1007/BF01570963
48. Wood S, Metcalf D, Devine D, Robinson C. Erythrosine is a potential photosensitizer for the photodynamic therapy of oral. J Antimicrob Chemother 2006; 57:680-4; PMID:16464894; http://dx.doi.org/10.1093/jac/dkl021
49. Abels C, Fickweiler S, Weiderer P, Bäumler W, Hofstädter F, Landthaler M, Szeimies RM. Indocyanine green (ICG) and laser irradiation induce photooxidation. Arch Dermatol Res 2000; 292:404-11; PMID:10994775; http://dx.doi.org/10.1007/s004030000147
50. Meyer M, Speight P, Bown SG. A study of the effects of photodynamic therapy on the normal tissues of the rabbit jaw. Brit J Cancer 1991; 64:1093-7; PMID:1764372; http://dx.doi.org/10.1038/bjc.1991.470
51. Ge L, Shu R, Li Y, Luo L, Song Z, Xie Y, Liu D. Adjunctive effect of photodynamic therapy to scaling and root planing in the treatment of chronic periodontitis. Photomed Laser Surg 2011; 29:33-7; PMID:21166588; http://dx.doi.org/10.1089/pho.2009.2727
52. Berakdar M, Callaway A, Eddin MF, Ross A, Willershausen B. Comparison between scaling-root-planing (SRP) and SRP/photodynamic therapy: six-month study. Head Face Med 2012; 8:12-27; PMID:22480188; http://dx.doi.org/10.1186/1746-160X-8-12
53. Kolbe MF, Ribeiro FV, Luchesi VH, Casarin RC, Sallum EA, Nociti FH Jr., Ambrosano GMB, Cirano FR, Pimentel SP, Casati MZ. Photodynamic therapy during supportive periodontal care: clinical, microbiologic, immunoinflammatory, and patient-centered performance in a split-mouth randomized clinical trial. J Periodontol 2014; 85:277-86; http://dx.doi.org/10.1902/jop.2014.130559
54. Wu J, Xu H, Tang W, Kopelman R, Philbert MA, Xi C. Eradication of bacteria in suspension and biofilms using methylene blue-loaded dynamic nanoplatforms. Antimicrob. Agents Chemother 2009; 53:3042-8; http://dx.doi.org/10.1128/AAC.01604-08
55. Perni S, Piccirillo C, Pratten J, Prokopovich P, Chrzanowski W, Parkin IP, Wilson M. The antimicrobial properties of light-activated polymers containing methylene blue and gold nanoparticles. Biomaterials 2009; 30:89-93; PMID:18838166; http://dx.doi.org/10.1016/j.biomaterials.2008.09.020
56. Chong H, Nie C, Zhu C, Yang Q, Liu L, Lv F, Wang S. Conjugated polymer nanoparticles for light-activated anticancer and antibacterial activity with imaging capability. Langmuir 2012; 28:2091-98; PMID:22054172; http://dx.doi.org/10.1021/la203832h
57. Soukos NS, Som S, Abernethy AD, Ruggiero K, Dunham J, Lee C, Doukas AG, Goodson JM. Phototargeting oral black-pigmented bacteria. Antimicrob Agents Chemother 2005; 49:1391-6; PMID:15793117; http://dx.doi.org/10.1128/AAC.49.4.1391-1396.2005
58. Cieplik F, Späth A, Leibl C, Gollmer A, Regensburger J, Tabenski L, Hiller KA, Maisch T, Scmalz G. Blue light kills *Aggregatibacter actinomycetemcomitans* due to its endogenous photosensitizers. Clin Oral Invest 2013; [Epub ahead of print].
59. Kuramitsu HK. Proteases of *Porphyromonas gingivalis*: what don't they do? Oral Microbiol Immunol 1998; 13:263-70; PMID:9807117; http://dx.doi.org/10.1111/j.1399-302X.1998.tb00706.x
60. Grenier D, Mayrand D. Selected characteristics of pathogenic and non-pathogenic strains of *Bacteroides gingivalis*. J Clin Microbiol 1987; 25:738-40; PMID:3571482
61. Batten MR, Senior BW, Kilian M, Woof JM. Amino acid sequence requirements in the hinge of human immunoglobulin A1 (IgA1) for cleavage by streptococcal IgA1 proteases. Infect Immun 2003; 71:1462-9; PMID:12595464; http://dx.doi.org/10.1128/IAI.71.3.1462-1469.2003
62. Grayson R, Douglas CWI, Heath J, Rawlinson A, Evans GS. Activation of human matrix-metalloproteinase 2 by gingival crevicular fluid and *Porphyromonas gingivalis*. J Clin Periodontol 2003; 30:542-550; PMID:12795793; http://dx.doi.org/10.1034/j.1600-051X.2003.00301.x
63. Andrian E, Mostefaoui Y, Rouabhia M, Grenier D. Regulation of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases by *Porphyromonas gingivalis* in an engineered human oral mucosa model. J Cell Physiol 2007; 211:56-62; PMID:17226791; http://dx.doi.org/10.1002/jcp.20894
64. Ingmer H, Brøndsted L. Proteases in bacterial pathogenesis. Res Microbiol 2009; 160:704-10; PMID:19778606
65. McGillivray SM, Ebrahimi CM, Fisher N, Sabet M, Zhang DX, Chen Y, Haste NM, Aroian RV, Gallo RL, Guiney DG, Friedlander AM, et al. ClpX contributes to innate defense peptide resistance and virulence phenotypes of *Bacillus anthracis*. J Innate Immun 2009; 1:494-506; PMID:20375606; http://dx.doi.org/10.1159/000225955
66. Travis J, Potempa J. Bacterial proteinases as targets for the development of second generation antibiotics. Biochem Biophys Acta 2000; 1477:35-50; PMID:10708847
67. Maeda H. Role of microbial proteases in pathogenesis. Microbiol Immunol 1996; 40:685-99; PMID:8981341; http://dx.doi.org/10.1111/j.1348-0421.1996.tb01129.x
68. Gusman H, Travis J, Helmerhorst EJ, Potempa J, Troxler RF, Oppenheim FG. Salivary histatin 5 is an inhibitor of both host and bacterial enzymes in periodontal disease. Infect Immun 2001; 69:1402-8; PMID:11179305; http://dx.doi.org/10.1128/IAI.69.3.1402-1408.2001
69. Giacometti COA, Ghiselli R, Orlando F, Kamysz W, D'Amato G, Mocchegiani F, Lukasiak J, Silvestri C, Saba V, Scalise G. Potential therapeutic role of histatin derivative P-113d in experimental rat models of *Pseudomonas aeruginosa* sepsis. J Infect Dis 2004; 190:356-64; PMID:15216473; http://dx.doi.org/10.1086/421913
70. Zhu J, Luther PW, Leng Q, Mixson AJ. Synthetic histidine-rich peptides inhibit Candida species and other fungi in vitro: role of endocytosis and treatment implications. Antimicrob Agents Chemother 2006; 50:2797-805; PMID:16870774; http://dx.doi.org/10.1128/AAC.00411-06
71. Mellgren RL. Calcium-dependent proteases: an enzyme system active at cellular membranes? Faseb J 1987; 1:110-5; PMID:2886390
72. Eichinger A, Beisel HG, Jacob U. et al, Crystal structure of gingipain R: an Arg-specific bacterial cysteine proteinase with a caspase-like fold. Embo J 1999; 18: 5453-62; PMID:10523290; http://dx.doi.org/10.1093/emboj/18.20.5453
73. Gomez JE, Birnbaum ER, Darnall DW. The metal ion acceleration of the conversion of trypsinogen to trypsin. Lanthanide ions as calcium ion substitutes. Biochem 1974; 13:3745-50; http://dx.doi.org/10.1021/bi00715a020
74. McGillivray SM, Tran DN, Ramados NS, Alumasa JN, Okumura CY, Sakoulas G, Vaughn MM, Zhang DX, Keiler KC, Nizet V. Pharmacological inhibition of the ClpXP protease increases bacterial susceptibility to host Cathelicidin antimicrobial peptides and cell envelope-active antibiotics. Antimicrob Agents Chemother 2012; 56:1854-61; PMID:22252821; http://dx.doi.org/10.1128/AAC.05131-11
75. Michel A, Agerer F, Hauck CR, Herrmann M, Ullrich J, Hacker J, Ohlsen K. Global regulatory impact of ClpP protease of *Staphylococcus aureus* on regulons involved in virulence, oxidative stress response, autolysis, and DNA repair. J Bacteriol 2006; 188:5783-96; PMID:16885446; http://dx.doi.org/10.1128/JB.00074-06
76. Capestany CA, Tribble GD, Maeda K, Demuth DR, Lamont RJ. Role of the Clp system in stress tolerance, biofilm formation, and intracellular invasion in *Porphyromonas gingivalis*. J Bacteriol 2008; 190:1436-46; PMID:18065546; http://dx.doi.org/10.1128/JB.01632-07
77. Potempa J, Pike R, Travis J. Titration and mapping of the active site of cysteine proteinases from *Porphyromonas gingivalis* (gingipains) using peptidyl chloromethanes. Biol Chem 1997; 378:223-30; PMID:9165075; http://dx.doi.org/10.1515/bchm.1997.378.3-4.223
78. Marsh PD, McDermid AS, McKee AS, Baskerville A. The effect of growth rate and haemin on the virulence and proteolytic activity of *Porphyromonas gingivalis* W50. Microbiology 1994; 140:861-5; PMID:8012602; http://dx.doi.org/10.1099/00221287-140-4-861

79. McKee AS, McDermid AS, Baskerville A, Dowsett AB, Ellwood DC, Marsh PD. Effect of haemin on the physiology and virulence of *Bacteroides gingivalis* W50. Infect Immun 1986; 52:349-55; PMID:3699884
80. Curtis MA, Aduse Opoku J, Rangarajan M, Gallagher A, Sterne JA, Reid CR, Evans HE, Samuelsson B. Attenuation of the virulence of *Porphyromonas gingivalis* by using a specific synthetic Kgp protease inhibitor. Infect Immun 2002; 70:6968-75; PMID:12438376; http://dx.doi.org/10.1128/IAI.70.12.6968-6975.2002
81. Maekawa T, Krauss JL, Abe T, Jotwani R, Triantafilou M, Triantafilou K, Hashim A, Hoch S, Curtis MA, Nussbaum G, et al. *Porphyromonas gingivalis* manipulates complement and TLR signaling to uncouple bacterial clearance from inflammation and promote dysbiosis. Cell Host Microbe 2014; 15:768-78; PMID:24922578; http://dx.doi.org/10.1016/j.chom.2014.05.012
82. Shinsuke Kataoka S, Baba A, Suda Y, Takii R, Hashimoto M, Kawakubo T, Asao T, Kadowaki T, Yamamoto K. A novel, potent dual inhibitor of Arg-gingipains and Lys-gingipain as a promising agent for periodontal disease therapy. FASEB J 2014 (Epub ahead of print, August 2014); http://dx.doi.org/10.1096/fj.14-252130
83. Meurman JH Probiotics: do they have a role in oral medicine and dentistry? Eur J Oral Sci 2005; 113:188-96; PMID:15953242; http://dx.doi.org/10.1111/j.1600-0722.2005.00191.x
84. Nase L, Hatakka S, Savilahti E, Saxelin M, Ponka A, Poussa T, Korpela R, Meurman JH. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* CG, in milk on dental caries and caries risk children. Caries Res 2001; 35:412-20; PMID:11799281; http://dx.doi.org/10.1159/000047484
85. Busscher HJ, Mulder AF, van der Mei CH. In vitro adhesion to enamel and in vivo colonization of tooth surfaces by lactobacilli from a bio-yogurt. Caries Res 1999; 33:403-4; PMID:10460966; http://dx.doi.org/10.1159/000016541
86. Calgar E, Cilder SK, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55739 by straws or tablets. Acta Odontol Scand 2006; 64:314-8; PMID:16945898; http://dx.doi.org/10.1080/00016350600801709
87. Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontol Scand 2005; 63:317-20; PMID:16512103; http://dx.doi.org/10.1080/00016350510020070
88. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased gum bleeding and reduced gingivitis by the probiotic *Lactobacillus reuteri*. Swed Dent J 2006; 30:55-60; PMID:16878680
89. Zahradnik RT, Magnusson I, Walker C, McDonell E, Hillman CH, Hillman JD. Preliminary assessment of safety and effectiveness in humans of ProBiora3, a probiotic mouthwash. J Appl Microbiol 2009 107:682-90; PMID:19486429; http://dx.doi.org/10.1111/j.1365-2672.2009.04243.x
90. Hillman JD, Brooks TA, Michalek SM, Harmon CC, Snope JL, van der Weijden CC. Construction and characterization of an effector strain of *Streptococcus mutans* for replacement therapy of dental caries. Infect Immun 2000; 68:543-9; PMID:10639415; http://dx.doi.org/10.1128/IAI.68.2.543-549.2000
91. Devine DA, Marsh PD. Prospects for the development of probiotics and prebiotics for oral applications. J Oral Microbiol 2009; 1:1; http://dx.doi.org/10.3402/jom.v1i0.1949
92. Coppa GV, Bruni S, Morelli L, Soldi S, Gabrielli O. The first prebiotics in humans: human milk oligosaccharides. J Clin Gastroenterol 2004; 38:S80-3; PMID:15220665; http://dx.doi.org/10.1097/01.mcg.0000128926.14285.25
93. Gorr S-U, Abdolhosseini M. Antimicrobial peptides and periodontal disease. J Clin Periodontol 2011; 38:126-41; PMID:21323710; http://dx.doi.org/10.1111/j.1600-051X.2010.01664.x
94. Allaker, RP. Host defence peptides—a bridge between the innate and adaptive immune responses. Trans R Soc Trop Med Hyg 2008; 102:3-4; PMID:17727907; http://dx.doi.org/10.1016/j.trstmh.2007.07.005
95. Handfield M, Mans JJ, Zheng G, Lopez MC, Mao S, Progulske-Fox A, Narasimhan G, Baker HV, Lamont RJ. Distinct transcriptional profiles characterize oral epithelium-microbiota interactions. Cell Microbiol 2005; 7:811-23; PMID:15888084; http://dx.doi.org/10.1111/j.k1462-5822.2005.00513.x
96. Wu Y, Shu R, Luo LJ, Ge LH, Xie YF. Initial comparison of proteomic profiles of whole unstimulated saliva obtained from generalized aggressive periodontitis periodontitis patients and healthy control subjects. J Perio Res 2009; 44:636-44; http://dx.doi.org/10.1111/j.1600-0765.2008.01172.x
97. Diamond G, Beckloff N, Weinberg A, Kisich KO. The roles of antimicrobial peptides in innate host defense. Curr Pharm Des 2009; 15:2377-92; PMID:19601838; http://dx.doi.org/10.2174/138161209788682325
98. Allaker RP, Zihni C, Kapas S. An investigation into the antimicrobial effects of adrenomedullin on members of the skin, oral, respiratory tract and gut microflora. FEMS Immunol Med Microbiol 1999; 23:289-93; PMID:10225288; http://dx.doi.org/10.1016/S0928-8244(98)00148-5
99. Lundy FT, O'Hare MM, McKibben BM, Fulton CR, Briggs JE, Linden GJ. Radioimmunoassay quantification of adrenomedullin in human gingival crevicular fluid. Arch Oral Biol 2006; 51:334-8; PMID:16226215; http://dx.doi.org/10.1016/j.archoralbio.2005.08.006
100. He J, Anderson MH, Shi W, Eckert R. Design and activity of a 'dual-targeted' antimicrobial peptide. Int J Antimicrob Agents 2009; 33:532-7; PMID:19188046; http://dx.doi.org/10.1016/j.ijantimicag.2008.11.013
101. He J, Yarbrough DK, Kreth J, Anderson MH, Shi W, Eckert R. Systematic approach to optimizing specifically targeted antimicrobial peptides against *Streptococcus mutans*. Antimicrob Agents Chemother 2010; 54:2143-51; PMID:20211885; http://dx.doi.org/10.1128/AAC.01391-09
102. Zasloff M. Antimicrobial peptides of multi-cellular organisms. Nature 2002; 415: 389-95; PMID:11807545; http://dx.doi.org/10.1038/415389a
103. Tew GN, Clements D, Tang H, Arnt L, Scott RW. Antimicrobial activity of an abiotic host defense peptide mimic. Biochim Biophys Acta 2006; 1758:1387-92; PMID:16626628; http://dx.doi.org/10.1016/j.bbamem.2006.03.001
104. Beckloff N, Laube D, Castro T, Furgang D, Park S, Perlin D, Clements D, Tang H, Scott RW, Tew GN, et al. Activity of an antimicrobial peptide mimetic against planktonic and biofilm cultures of oral pathogens. Antimicrob Agents Chemother 2007; 51:4125-32; PMID:17785509; http://dx.doi.org/10.1128/AAC.00208-07
105. Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiol 2003; 149:279-4; http://dx.doi.org/10.1099/mic.0.26082-0
106. Chung WO, Dommisch H, Yin L, Dale BA. Expression of defensins in gingiva and their role in periodontal health and disease. Curr Pharm Design 2007; 13: 3073-83; http://dx.doi.org/10.2174/138161207782110435
107. Allaker RP, Ren G. Potential impact of nanotechnology on the control of infectious diseases. Trans R Soc Trop Med Hyg 2008; 102:1-2; PMID:17706258; http://dx.doi.org/10.1016/j.trstmh.2007.07.003
108. Allaker RP. The use of nanoparticles to control oral biofilm formation. J Dental Res 2010; 89: 1175-86; PMID:20739694; http://dx.doi.org/10.1177/0022034510377794
109. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol 2008; 35:286-91; PMID:18724856; http://dx.doi.org/10.1111/j.1600-051X.2008.01274.x
110. Giersten E. Effects of mouth rinses with triclosan, zinc ions, copolymer, and sodium lauryl sulphate combined with fluoride on acid formation by dental plaque in vivo. Caries Res 2004; 38:430-35; PMID:15316186; http://dx.doi.org/10.1159/000079623
111. Sondi I, Salopek-Sondi B. Silver nanoparticles as an antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 2004; 275:177-82; PMID:15158396; http://dx.doi.org/10.1016/j.jcis.2004.02.012
112. Cioffi N, Torsi L, Ditaranto N, Tantillo G, Ghibelli L, Sabbatini L, Bleve-Zacheo T, D'Alessio M, Zambonin PG, Traversa E. Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. Chem Mater 2005; 17:5255-62; http://dx.doi.org/10.1021/cm0505244
113. Boldyryeva H, Umeda N, Plaskin OA, Takeda Y, Kishimoto N. High-fluence implantation of negative metal ions into polymers for surface modification and nanoparticle formation. Surf Coat Tech 2005; 196:373-77; http://dx.doi.org/10.1016/j.surfcoat.2004.08.159
114. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir 2002; 18:6679-86; http://dx.doi.org/10.1021/la0202374
115. Karlsson HL, Cronholm P, Gustafsson J, Moller L. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem Res Toxicol 2008; 21:1726-32; PMID:18710264; http://dx.doi.org/10.1021/tx800064j
116. Memarzadeh K, Sharili AS, Huang J, Rawlinson SC, Allaker RP. Nanoparticulate zinc oxide as a coating material for orthopaedic and dental implants. J Bio Mat Res A in press; http://dx.doi.org/10.1002/jbm.a.35241
117. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. Appl Environ Microbiol 2007; 73:1712-20; PMID:17261510; http://dx.doi.org/10.1128/AEM.02218-06
118. Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weiss EI. Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against *Streptococcus mutans*. Biomaterials 2006; 27:3995-4002; PMID:16564083; http://dx.doi.org/10.1016/j.biomaterials.2006.03.003
119. Stephen KW. Dentrifices: recent clinical findings and implications for use. Int Dent J 1993; 43:549-53; PMID:8138326
120. Waltimo T, Brunner TJ, Vollenweider M, Stark WJ, Zehnder M. Antimicrobial effect of nanometric bioactive glass 45S5. J Dent Res 2007; 86:754-757; PMID:17652205; http://dx.doi.org/10.1177/154405910708600813
121. Wu Y, Yang W, Wang C, Hu J, Fu S. Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. Int J Pharmac 2005; 295:235-45; http://dx.doi.org/10.1016/j.ijpharm.2005.01.042
122. Venegas SC, Palacios JM, Apella MC, Morando PJ, Blesa MA. Calcium modulates interactions between bacteria and hydroxyapatite. J Dent Res 2006; 85:1124-28; PMID:17122166; http://dx.doi.org/10.1177/154405910608501211
123. Rahiotis C, Vougiouklakis G, Eliades G. Characterization of oral films formed in the presence of a CPP-ACP agent: an in situ study. J Dent 2008; 36:272-80; PMID:18291571; http://dx.doi.org/10.1016/j.jdent.2008.01.005
124. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing

gum. J Dent Res 2003; 82:206-11; PMID:12598550; http://dx.doi.org/10.1177/154405910308200311
125. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? Aus Dent J 2008; 53:268-73; http://dx.doi.org/10.1111/j.1834-7819.2008.00061.x
126. Nel A, Xia T, Madler I, Li N. Toxic potential of materials at the nanolevel. Science 2006; 311:622-27; PMID:16456071; http://dx.doi.org/10.1126/science.1114397
127. Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater 2009; 8:543-57; http://dx.doi.org/10.1038/nmat2442
128. Seetharam RN, Sridhar KR. Nanotoxicity: threat posed by nanoparticles. Curr Sci 2006; 93:769-70.
129. Nair S, Sasidharan A, Rani VVD, Menon D, Nair S, Manzoor K, Raina S. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J Mater Sci Mater Med 2009; 20:S235-S41; PMID:18716714; http://dx.doi.org/10.1007/s10856-008-3548-5