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Biological approaches to the development of novel periodontal therapies – Consensus of the Seventh European Workshop on Periodontology

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Abstract

Background: Periodontitis remains a major public health issue and current management approaches have failed to impact upon the most high-risk proportion of the population and those with the most severe disease. The objective of this session was to assess if and how, current understanding of periodontitis provides the opportunity to develop new preventive and therapeutic strategies.

Materials and Methods: Based on the current understanding of the pathophysiology of periodontal diseases, the Workshop discussed the potential of antimicrobial peptides, probiotics, pro-resolving lipid mediators, and micronutritional approaches. Evidence-based position papers and expert discussions formed the basis of deliberations.

Results and Discussion: Current preventive and treatment approaches are only partially effective, and this appears due to the therapeutic focus remaining primarily upon biofilm management rather than embracing a pivotal role for inflammation as a driver of biofilm composition as well as tissue damage. There is a need to develop new, more effective, and efficient preventive and treatment approaches for gingivitis and periodontitis, which embrace recent advances in understanding of host modulation and inflammation resolution, as well as direct management of the microbiota.

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The goal of successful periodontal care is prevention and treatment of periodontal inflammation and establishment of a beneficial or health-associated biofilm. For the majority of the population, preventive and treatment approaches based on biofilm control are partially effective (less gingivitis, less moderate periodontitis, greater tooth retention). Limitations of current strategies include access to care and adherence to professional advice. In addition, these

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approaches alone are not sufficient for a significant minority of the population that is highly susceptible to periodontitis. Evidence is emerging that the inflammatory response influences the composition of the biofilm; hence, control of inflammation may prevent the emergence of a pathogenic biofilm and promote healing. Furthermore, the individual inflammatory response is governed by modifiable (lifestyle-associated) and non-modifiable (genetic) exposures.

Given current limitations, new approaches to controlling inflammation are required.

The new therapeutic approaches that will be discussed are divided into two major, albeit overlapping categories: modification of the biofilm and modification of the host response.

Antimicrobial Peptides (AMP) (Gorr & Abdolhosseini 2011)

What are the physiological roles of AMP?

AMPs can affect the homeostasis of the oral cavity through broad or selective killing of bacteria, affecting colonization, exhibiting anti-inflammatory activity and binding to bacterial toxins or immune modulation. AMPs can effect cell migration and link innate and adaptive immunity via dendritic cell activation. It is likely that AMPs work in a combination that has effects against individual bacterial species and may have additive effects. Additional physiological effects, including wound healing properties and cytotoxicity, have been proposed but remain controversial (Lehrer 2004).

What is the evidence that AMP deficiencies may underpin periodontal inflammation?

The expression profiles of AMPs are differentially affected by patterns of bacterial challenge. Evidence is emerging that direct AMP deficiencies could play a role in periodontal disease. As examples, the genetic diseases Morbus Kostman, Papillon-Lefevre, Haim-Munk, exhibit deficiency of LL-37 or conversion of cathelicidin to active LL-37 that is associated with periodontitis. Similarly, variations in the hBD1 promoter region are associated with periodontitis (Kalus et al. 2009, Schaefer et al. 2010).

What are the AMP concentrations at the site of action and are they sufficient for preventive or therapeutic effects?

The effective concentrations of AMPs and their sites of action have yet to be identified. Several AMPs are present in saliva and GCF; the major sources are gingival epithelia, neutrophils and salivary glands. A full bacterial target and susceptibility profile is not available in most cases. The concentrations in these fluids are lower than the minimal inhibitory concentrations for some bacteria and higher for others. It is possible that the AMPs in oral fluids exert antibacterial effects. Conversely, AMPs may not function directly in saliva or GCF but rather at the gingival epithelial cell surface or at the site of secretion from neutrophils where the local concentration could be higher than that found in the oral fluids.

What is the evidence that AMPs can be used to selectively modify biofilm composition and is there potential for a preventive or therapeutic benefit?

At present there is no evidence that AMPs can selectively modify biofilm composition. The potential exists for selective modification of biofilm bacteria. Future research should address whether individual AMPs have differential activities against different bacteria and if individual differences in gene copy number and magnitude of expression influence the levels of individual AMPs. It should be determined whether AMP expression patterns/levels can be stimulated pharmacologically or nutritionally and if AMP expression patterns change during different activity phases of the disease.

What are the potential approaches for using AMPs for prevention and for treatment of periodontitis?

It is too early to recommend specific approaches for prevention and treatment. The prevention of periodontitis could depend upon antibacterial, anti-inflammatory and/or immune modulatory actions of AMPs. Approaches that stimulate or restore the natural expression patterns of AMPs, e.g. receptor activation or inhibition, may be most successful for prevention of disease. Additional studies should determine whether bacterial or endogenous protease inhibitors can enhance the activity or longevity of AMPs or related receptors (PAR2) (Chung et al. 2007). It is anticipated that treatment of periodontal disease by AMPs will be most successful in conjunction with mechanical debridement.

What are the potential detrimental effects from elevated levels of certain AMPs?

At this time it is not possible to define what elevated levels of AMPs are due to a lack of knowledge of the normal concentrations and the site of action in most cases. However, AMPs can be pro-inflammatory in epithelial cells and may enhance migration of mast cells (Niyonsaba et al. 2007). Application of individual AMPs as therapeutic agents could cause bacterial resistance and may be associated with host toxicity, e.g. hemolysis.

In relation to the potential use of probiotics it should be determined whether AMPs kill or modulate the activity of probiotics and, conversely, if probiotics affect the expression of AMPs and/or secrete proteases that degrade AMPs.

Probiotics (Teughels 2011)

What are the characteristics of a ''health-associated'' or beneficial biofilm?

We currently consider a biofilm to be host-compatible when it contains relatively high proportions of Gram-positive, facultative anaerobic bacteria and low proportions of Gram-negative, anaerobic species and is associated with absence of inflammation. To date, few cultural and/or molecular microbiological studies have focused on oral health so the composition of the health-associated microbiota remains poorly characterized. Individual variability is a predominant feature of health-associated microbiota. The composition of the biofilm is not only dependent upon its microbiological content but also on the host's immunological status and local environmental factors. *From the periodontal perspective it is the host and not the biofilm that should be considered ''healthy''.*

What is the evidence that probiotics offer potential for oral biofilm manipulation and improved clinical outcomes?

Limited data from pilot studies suggest that probiotics have the potential to modify, at least in the short term, the oral microbiota by either direct

microbiological interactions or by immune modulatory interactions. At present, clinical benefits have not been demonstrated.

Which probiotic strains are appropriate for prevention and treatment of periodontal diseases?

It is currently not possible to specify which species are the most appropriate for periodontal health or to extrapolate findings from other fields (e.g. gastrointestinal) to periodontal healthcare. Therefore, more studies on the composition of the oral health-associated biofilm need to be performed and organisms that are shown to be associated with health and not disease could be screened for their potential value as probiotics. Pipeline screening methods could be used to look for inhibitory activity against disease-associated species as well as for the presence of any virulence factors or antimicrobial resistance properties which might prevent their use. Similar screenings may be used to identify possible immune-modulatory mechanisms.

What is the relative importance of direct antimicrobial and host-modulatory probiotic mechanisms in prevention and therapy of periodontal diseases?

Studies on the effect of probiotics on periodontal health have primarily focused on the alteration of the microbiota. It is well established, however, that probiotics also demonstrate anti-inflammatory properties. As in other fields of probiotic research, it is currently unclear which of these mechanisms of action is important and further research is warranted. Furthermore, the antimicrobial and anti-inflammatory effects of probiotics are strain specific and to achieve optimal effects, simultaneous use of multiple species may be warranted.

Pro-resolving Lipid Mediators (Van Dyke 2011)

What is the evidence that control of inflammation results in reduction/elimination of pathogens?

It has been demonstrated in the rabbit periodontitis model that control of inflammation resulted in the spontaneous elimination of ''periodontal pathogens'' (e.g. *Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans*). This question is central to the cause/effect relationship of specific bacteria and periodontitis. In one human study in young Indonesian adults followed for 15-years (van der Velden et al. 2006) *A. actinomycetemcomitans* colonization preceded attachment loss. However, in another human study (Tanner et al. 2007), a series of young subjects with little or no periodontitis was followed longitudinally over 18-months with the goal of determining if a specific organism or group of organisms emerged before attachment loss. The presence of a specific organism or group of organisms before attachment loss or the overgrowth of any specific organism or group of organisms did not predict attachment loss. However, gingivitis did predict future attachment loss, which is further supported by the long-term follow-up of the Oslo cohort demonstrating that the degree of gingivitis predicts long-term tooth loss (Schätzle et al. 2004, Lang et al. 2009). Thus while there is some evidence in aggressive periodontitis that *A. actinomycetemcomitans* colonization may precede attachment loss (Fine et al. 2007, Haubek et al. 2008), there is no evidence that species associated with chronic periodontitis predict attachment loss, whereas gingival inflammation is a pre-requisite.

The literature supports the concept that inflammation impacts upon biofilm composition. Recent studies reveal that the biofilm associated with IBD lesions forms because of the inflammatory lesion and the overgrowth of *Helicobacter pylori* in gastric ulcers is driven the ulcer formation. These concepts are consistent with the modern understanding of biofilm/host dynamics (Marsh 2011) discussed in Group 1 of these proceedings.

These data are preliminary and far from conclusive; however, the suggested concept has the potential to change the treatment paradigm for inflammatory periodontal diseases induced by oral biofilms. Further detailed research investigating the potential for host modulatory therapy as the primary target for control of periodontitis is required.

What is the evidence that there is a failure of resolution of inflammation pathways in gingivitis and periodontitis patients?

Aggressive periodontitis represents a group of diseases where chronic activation of inflammatory cells seems to be exaggerated. While early literature interpreted observations of reduced chemotaxis and phagocytosis as deficiencies or defects in the innate immune response, it is now realized that these observations are the result of chronic pre-activation or ''priming'' of inflammatory cells that also result in increased release of tissue destructive enzymes and reactive oxygen species. Investigation of the hyper-responsive LAP PMN in vitro revealed that these cells are refractory to the pro-resolving lipoxins, but not the resolvins. This single observation is supported by indirect evidence in humans and animals, which demonstrates that addition of pro-resolving agents in periodontitis has a positive therapeutic impact.

What are the potential risks associated with using pro-resolving mediators in periodontitis prevention or treatment?

Resolution of inflammation is not inhibition of inflammation. Pro-resolving mediators enhance bacterial clearance while limiting tissue damage. In a sepsis model (caecal ligation and puncture), administration of RvD1 reduced lethality and increased bacterial clearance (Spite et al. 2009). To date, the weight of the evidence in a variety of animal models suggests that super-infection is an unlikely complication. However, more investigations are necessary to determine the optimal dose of these agents, whether there is tissue specificity for their actions, and whether chronic use of resolving agents will result in cell desensitization and resistance.

What are the potential challenges associated with using pro-resolving molecules in clinical practice?

It is too early to speculate on the intended use as a periodontal therapeutic. It is doubtful that an inflammation-modulating agent will ever completely replace some form of mechanical therapy aimed at the control of the biofilm. If the veracity of the hypothesis that the inflammation impacts upon the composition of the biofilm is proven, the biofilm will still be there. Reduction of the mass of the biofilm as a means of reducing the inflammatory burden remains a viable approach. The use of these molecules may be an interesting preventive approach that will protect against the acquisition or overgrowth of intrinsically pathogenic bacteria.

Should we expect a benefit from any anti-inflammatory agent? What anti-inflammatory regimen is available today?

There is an extensive literature demonstrating that anti-inflammatory agents such as Flurbiprofen can alter the course of periodontal disease in animals and humans (for a review, see Reddy et al. 2003). Our understanding of the actions of COX inhibitors has matured considerably in recent years. It is now appreciated that NSAIDs in general are ''resolution toxic''. Briefly, the PGE2 response, which is the target of NSAIDs, is the cellular signal for the initiation of resolution pathways. Hence, blocking the PGE2 response blocks the resolution response creating chronic inflammation without pain. In rheumatoid arthritis, chronic administration of COX inhibitors contributes to worsening and more rapid progression of the arthritis lesions (Chan and Moore 2010).

In the context of periodontitis and the observations with Flurbiprofen, the short-term outcome is inhibition of periodontal bone loss. However, withdrawal of the drug resulted in a rebound of disease and chronic administration of the drug is impossible due to severe side effects.

Since pro-resolution agonists are early in development and not available for human use, the question of whether dietary macronutrient (e.g. omega-3 PUFAs) approaches to increasing pro-resolving agonists is a viable alternative. A pilot study indicated that n-3 PUFA and 81 mg aspirin had a positive adjunctive clinical outcome for PPD and CAL.

Nutritional modulation of periodontal inflammation (Van der Velden et al. 2011)

What is the evidence that diet may cause inflammation?

There is evidence from both association, cohort and intervention studies that increased caloric intake induces inflammation directly via glucose metabolic and signalling pathways (post-prandial oxidative stress) (Monnier et al. 2006, Ceriello et al. 2008, Esposito et al. 2008) and indirectly through visceral fat accumulation (adiposity) (O'Keefe & Bell 2007, O'Keefe et al. 2008). Caloric restriction will reduce clinical and biological measures of inflammation (Reynolds et al. 2009).

Increased central adiposity is associated with increased periodontitis prevalence (Pischon et al. 2007). There is evidence that increased intake of refined sugars leads to increased gingival bleeding and removal of refined sugars from the diet reduces gingival bleeding (Sidi & Ashley 1983, Baumgartner et al. 2009).

What is the evidence that specific nutrient deficiencies are associated with periodontitis?

Association studies demonstrate an inverse relationship between intake of fibre (Merchant et al. 2006), antioxidant micronutrients and polyunsaturated fatty acids and periodontitis prevalence (Jenzsch et al. 2009, Chapple et al. 2010). There is also an inverse relationship between plasma levels of vitamin D, vitamin C, magnesium, and combined small molecule antioxidants and periodontitis.

What is the evidence that dietary recommendations involving reductions in certain food types are effective in preventing/treating periodontal diseases?

Dietary recommendations for reducing caloric intake and refined sugars are part of mainstream medical advice where there is evidence from the history and anthropometrics that reductions are indicated. A similar approach for managing inflammatory periodontal diseases may be desirable, but robust evidence for this approach is currently lacking. Further intervention studies in this area are recommended.

What is the evidence that periodontal outcomes may be improved by nutritional supplements?

At present there is a body of evidence from cross-sectional studies that support the potential to improve periodontal outcomes by using dietary antioxidant phytonutritional interventions and by vitamin D supplementation in patients with vitamin D deficiency. However, clearly there is a need for longitudinal and intervention studies in order to provide robust evidence for this approach.

What is the need to adopt a population-based nutritional strategy to periodontal prevention?

There is a need to encourage governments to introduce/reinforce strategies to improve the nutritional content of food products as part of a strategy to reduce chronic inflammatory diseases, specifically in relation to fibre, refined sugar, omega-3 PUFA and certain antioxidant micronutrient contents. Such an approach may also be beneficial for the prevention of periodontal disease, but further evidence from research must be provided.

Dietary recommendations for prevention and treatment of periodontal inflammation based upon current evidence

Placing periodontal health within the context of general health, there may be benefit from the dental team incorporating advice to increase dietary intake of fibre, fish oils, fruits, vegetables and berries, and reduce intake of refined sugars as part of periodontal prevention and treatment strategies for general health, where appropriate. In obese patients restriction of caloric intake should also be advised. Evidence is starting to emerge that such an approach may also provide benefit to periodontal health.

Conclusions

The European Workshop on Periodontology participants agreed that:

- Periodontitis remains a major public health issue and current management approaches have failed to impact upon the most high-risk proportion of population and those with the most severe disease.
- Current preventive and treatment approaches are only partially effective, and this appears due to the therapeutic focus remaining primarily upon biofilm management rather than embracing a pivotal role for inflammation as a driver of biofilm composition as well as tissue damage.
- There is a need to develop new, more effective and efficient preventive and treatment approaches for gingivitis and periodontitis, which embrace recent advances in understanding of host modulation and inflammation resolution, as well as direct management of the microbiota.
- Such approaches need to be based upon emerging understanding of the pathobiology of periodontal diseases

and robust randomized-controlled clinical trials of novel therapeutics.
- Promising approaches include those discussed in this Workshop.
- In spite of significant progress, important limitations in the understanding of periodontal diseases remain and have an impact upon the development of new approaches to treatment and prevention.
- Translation requires significant additional research efforts that should be supported by public and industry funds.

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