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Krill Enzymes (Krillase®) an Important Factor to Improve Oral Hygiene

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1. Introduction

Prevention of gingivitis is largely governed by limiting the development of oral dental plaque and biofilm formation. It is also broadly acknowledged that accumulation of microorganisms are of pivotal importance in the initiation and progression of gingivitis and associated oral diseases (Socransky, Haffajee, 2005). We propose that krill enzymes (Krillase®) as they disintegrate cell surface structures and diminish bacterial adhesion to be a novel and innovative method for prevention of gingivitis.

Krillase® is isolated from the digestive tract of Antarctic krill (*Euphausia superba*), a shrimp like animal constituting an enormous biomass in the Antarctic convergence. The harsch ecological situation in the Antarctica implies that krill has an exceptionally effective digestive apparatus containing a co-operative multi-enzyme system involving both endo- and exopeptidases. Moreover, these enzymes have much lower activation energies than those of mammalian enzymes ensuring fast and highly efficient breakdown of diverse biological substrates (Hellgren et al, 1999).

The objective for the development of Krillase® has been to maintain the natural composition of krill enzymes intact throughout the purification process. Krillase® is defined as a mixture of acidic endopeptidases (trypsin- and chymotrypsin-like enzymes) and exopeptidases (carboxypeptidase A and B).

The final product (chewing gum) is well characterized with respect to stability, enzyme activity, uniformity and biocompability. Data from toxicology, pharmacology, pre- resp clinical studies give evidence for a broad safety profile.

Krill enzymes, due to their unique synergistic action, have been proven to exert both quantitative and qualitative effects on dental plaque/biofilm as well as on bacterial adherence to teeth surfaces. This leads to significant decrease in plaque accumulation and reduction in occurrence of gingivitis and caries pathogenesis (Hellgren, 2009).

In summary, Krillase® constitutes an important future alternative to a variety of other more toxic chemicals presently marketed for oral use including bisguanid, triclosan, aminoalcohols.

2. Clinical studies

In our recent study the effect of Krillase® chewing gum on gingival inflammation and dental plaque formation was investigated (Hellgren, 2009). Ten healthy volunteers aged 21-

45 (average age 22.4 yrs) chewed Krillase for 10 minutes after each ?meal in conjunction with normal oral hygiene measures. The test chewing gums contained either 6.0 or 0.06U of Krillase ® versus placebo, and were chewed four times a day during a 10 day test period. In a double-blind cross-over design, each participant concluded three consecutive trial periods for each gum The severity of gingival bleeding was measured by probing gingival pockets at selected teeth including first molar and forward in each jaw, initially and after each test period. In parallel a plaque index was established.

A therapeutic dose of 0.06U Krillase in the chewing gum reduced the mean gingival bleeding index by 54% compared to baseline ($p < 0.05$) and significantly better ($p < 0.05$) than the placebo gums reduction of 21% compared to baseline. Also the Mean Simplified Debris Index which measures (Green, Vermillion, 1964) decreased by 60% from its initial status for the 0.06U Krillase® gum ($p < 0.05$) and 14% as compared to placebo gum. The high dose (Krillase®, 6.0U) did not improve the efficacy. There was no significant statistical difference between the Krillase® gums and placebo on the Simplified Debris Index. None of the subjects reported any adverse reactions or events during the entire trial period. The chewing gum was reported to be neutral in taste. This study verified that a process of pathogenic plaque formation is disturbed by Krillase®, possibly via disruption of oral biofim and affecting adhesive properties of the oral bacteria. This, results in a numerical decrease of plaque formation and a significant reduction of gingival bleeding compared to a placebo chewing gum.

Krillase® profile and mode of action is well adopted to improve gingival health. These clinical data clearly illustrate the superiority of Krillase® compared to placebo gums.

Placebo gums, however, also diminish the number of gingival bleeding sites, something that might be associated both to the mechanical effect of chewing gum as well as to the known placebo phenomenon like unconscious awareness of oral hygiene of study participants.

These findings are in adherence with previous in pre- and clinical studies demonstrating that Krillase® disintegrates bacterial surface adhesive proteins and hampers colonization of dental surfaces (Hahn Berg, 2003).

In another study (Hellgren, unpublished data) we demonstrated that a less voluminous plaque and bleeding were observed on teeth treated with Krillase® gum than placebo both in short (5 days) and long term (14 days) perspective.

The patients in this double-blind crossover study had initially healthy gingival condition provided by professional cleansing in dental office in conjunction with thorough oral hygiene instructions. Thereafter each patient was given chewing gum (Krillase® gum or placebo gum) to be chewed five times a day for 10 min after the meals. During the test period of 5 or 14 days, the patients were instructed to eat normally but to restrain from any other oral hygiene measures apart from the prescribed chewing gums. After the first test period the baseline is restored and the patient started with the test period 2. The same procedure is then repeated for the third test period. Plaque index (PLI), gingival bleeding (BOP) and photographs were performed after each the test period and compared with the baseline values (Löe, Sillnes, 1963; Löe et al 1965; Löe1967).

At the end of each test period (day 5 or 14) the teeth's were dyed with Diaplaque® (coloring plaque as red), clinical evaluations as well as photographing were performed.

In addition to the usual and somewhat subjective clinical evaluations performed by independent observers such as dentists or hygienists, we also included a quantitative

imaged based assay in this study. Clinical evaluations are normally both time consuming and subjective, therefore further evaluations were performed by advanced computerized image analyses to objectively quantify the color differences in plaque formation and extension in the test photographs. To normalize all photographs before analysis a reference mm/gray scale in every photograph was used as an internal standard. Based on the reference scale the photos are corrected both for the geometric and photometric distortions. Thus, the reference gray scale in the photos makes it possible to recreate the original color reflectance values in each pixel in spite of variations in color temperature of the illumination, photographic emulsion and other factors, which cannot be fully controlled. The resulting data gave an accurate account of plaque extension in each particular tooth. Both subjective observer based evaluations and objective computerized analyses of standardized clinical photographs confirmed that patients chewing Krillase® chewing gum clearly formed less plaque than when chewing placebo gums after in 5 as well as 14 days trials. Some results are shown in **Figures 1-2**.

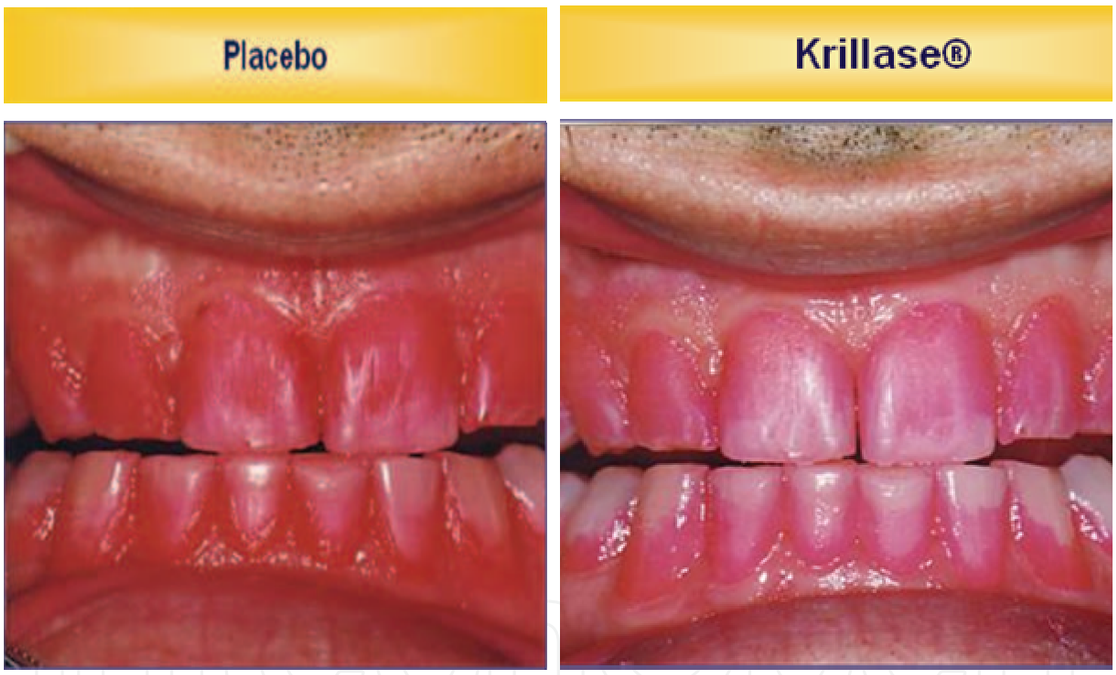


Fig. 1. Dental plaque extension after 14 days of treatment

Dental plaque development is closely associated with gingivitis and caries. Krillase® broad enzyme specificity in combination with its low autolysis warranted further studies on microbial adhesion in the oral cavity. SEM on *in vitro* plaque visualized that Krillase® significantly reduced the number of adhering microorganisms (Hahn Berg et al, 2001). These findings were further confirmed by elipsometry (Hahn Berg, 2003) data. The observed modifications of salivary and microbial proteins by Krillase® resulted in overall decrease of oral microflora abilities to attach. These findings are also supported by *in vivo* plaque study where Krillase® clearly detached microorganism from plaque accumulated on dentures (Hahn Berg, 2003).

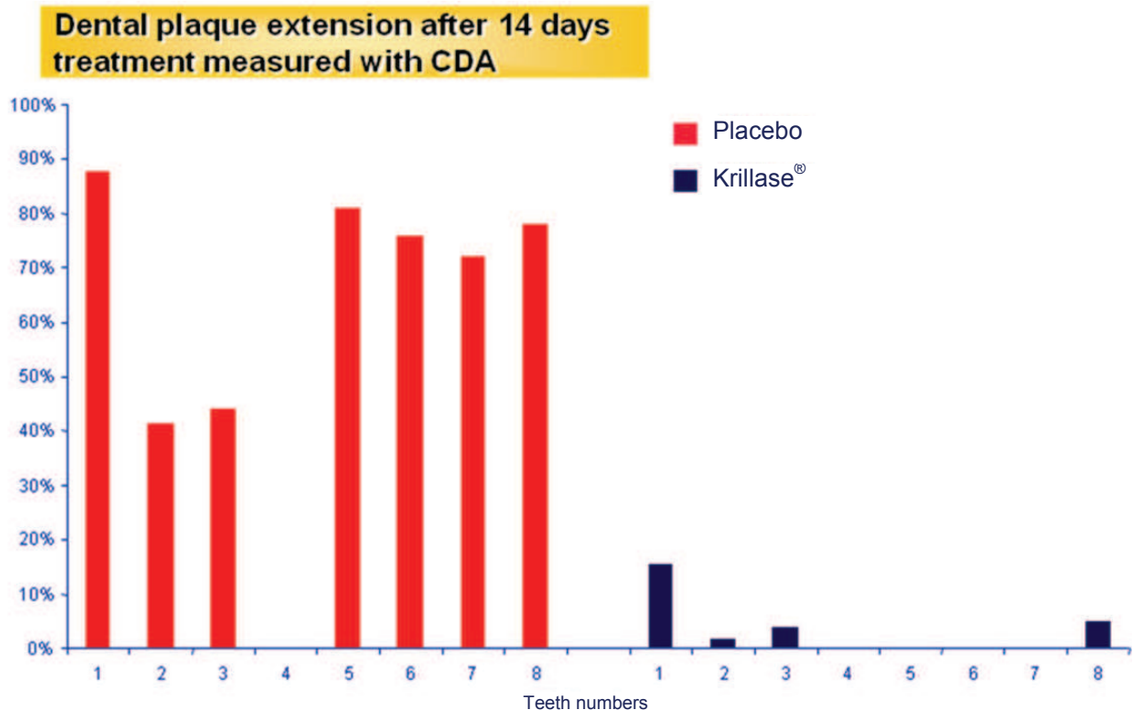


Fig. 2. Dental plaque extension after 14 days of treatment measured with CDA

Furthermore, our results also reveals that dental plaque exposed to carbohydrates in the presence of Krillase® looses most of its ability to produce acids causing the characteristic pH drop. The mechanism behind this phenomenon is unknown, but merits further and future investigations.

Another important observation is that krill enzymes besides counteracting bacterial adhesion does not alter the normal oral microflora, in contrast to the action of chlorhexidine or antibiotics. This is important since the current opinion is that oral ecosystem should not be altered by dental products. By disintegrating cell surface protein structures and in this way limiting bacterial adhesion, Krillase® treatment results in significant reduction of plaque accumulation and improves gingivits.

3. Discussion

Microorganisms continuously colonize oral surfaces forming initially dental plaque. The toxic products from plaque bacteria, like lactic acid from digested carbohydrates, induce local inflammation, gingivitis, periodontitis leading to caries and ultimately to tooth loss. Mechanical removal of plaque is still the most efficient way to prevent its accumulation and consequently development of dental diseases. A number of agents such as desinfectants/antiseptica (chlorhexidine, triclosan, herbal extracts), surfactants (sodium lauryl sulphate), sugar substitutes (xylitol) and enzymes (dextranases) are used for prevention against caries.

We have shown that Krillase® disruption of dental biofilm (pellicle) associated with diminished bacterial adherence to oral surfaces successfully prevents plaque formation and

gingival inflammation. These findings are supported by a series of both *in vitro* data as well as clinical studies.

A therapeutic dose of 0.06U of Krillase® was sufficient as complement to normal oral hygiene. A dramatic reduction in number of gingival bleeding sites as well as the plaque index was noted when chewing Krillase® gums were compared to initial status. The higher dose does not seem to improve the efficacy, in line with our earlier *in vitro* observations pointing to a substrate-enzyme dependency.

Another study further corroborate these results where less plaque and bleeding were observed on teeth treated with Krillase® gum compared to a placebo gum both in short (five days) and longer term (14 days). Krillase® plaque retarding effect was documented in patients with baseline washout using professional cleaning and the refraining from any oral hygiene for 10-14 days.

These clinical findings confirm the previous observations demonstrating that Krillase® efficiently remove fimbriae from the cell surface of plaque bacteria as well as detach them from dentures worn by patients.

To conclude, we propose that regular chewing Krillase® gum represent a novel and valid strategy to prevent plaque formation and gingivitis consequently improving general oral hygiene. Being highly biocompatible and with no side effects, Krillase® constitutes a promising candidate for modern preventive oral care.

4. References

[1] Socransky SS, Haffajee AD, Periodontal microbiology ecology, *Periodontal 2005; 38: 135-187*

[2] Hellgren L, Karlstam B, Mohr V, Vincent J, Peptide hydrolases from Antarctic krill – an important new tool with a promising medical potential, *In Biotechnological applications of cold-adapted organisms, p 63-74; eds R Margesin, F Schinner, Springer Verl Berlin Heidelberg 1999*

[3] Hellgren K Assessment of Krillase® chewing gum for the reduction of gingivitis and dental plaque, *J Clin Dent 20:99-102, 2009*

[4] Hellgren K, Efficacy of Krillase® chewing gum on gingivitis and dental plaque formation, *Unpublished data*

[5] Greene JC, Vermillion JR, The simplified oral hygiene index, *J Amer Dent Assoc 68: 7-13, 1964*

[6] Löe H, Silness J, Periodontal disease in pregnancy: I. Prevalence and severity, *Acta Odontologica Scand 21: 533-551, 1963*

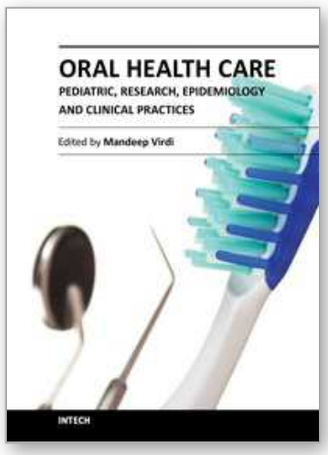
[7] Löe H, Theilade E, Jensen SB, Experimental gingivitis in man, *J Periodont 36: 177-187, 1965*

[8] Löe H, Oral hygiene in the prevention of caries and periodontal disease, *Int Dent J 50: 129-139, 2000*

[9] Löe H, The gingival index, the plaque index and the retention index systems, *J Periodontol 38: 610-616, 1967*

[10] Hahn Berg C, Kalfas S, Malmsten M, Arnebrant T, Proteolytic degradation of oral biofilms *in vitro* and *in vivo*: potential of proteases originating from *Euphausia superba* for plaque control, *Eur J Oral Sci 109: 316-324, 2001*

[11] Hahn-Berg CI, Properties of interfacial proteinaceous films with emphasis on oral systems, *PhD thesis, Inst Surface Chem, Stockholm and Dept Food Technol, Lund University, Lund, 2003*



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Oral health care in pediatric dentistry deals with complete oral health, including preventive aspects for children right from their conception to adolescence, encompassing all the spheres of dentistry including various specialties. It also includes planning a preventive program at individual and community levels. The current research interests in oral health care include studies regarding the role of stem cells, tissue culture, and other ground-breaking technologies available to the scientific community in addition to traditional fields such as anatomy, physiology, and pharmaceuticals etc of the oral cavity. Public health and epidemiology in oral health care is about the monitoring of the general oral health of a community, general afflictions they are suffering from, and an overall approach for care and correction of the same. The oral health care-giver undertakes evaluation of conditions affecting individuals for infections, developmental anomalies, habits, etc. and provides corrective action in clinical conditions. The present work is a compendium of articles by internationally renowned and reputed specialists about the current developments in various fields of oral health care.

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