CHITOSAN – ANTIBACTERIAL USE IN DENTAL MATERIALS

Secondary caries is the primary reason for replacement of composite fillings, and clinical studies report more secondary caries when composites are used compared to amalgam in high risk caries groups. This highlights the need for development of materials that will reduce or inhibit biofilm formation on dental materials.

The challenges of antibacterial agents in dental materials can be many:
• The agent must be present in a concentration that inhibits or reduce biofilm formation.
• The concentration must not exceed cytotoxic level.
• The aesthetic, mechanical and chemical qualities of the material must not deteriorate.
• The effect should not be limited in time.

We are currently investigating the inhibitory effect of chitosan on biofilm formation; an interesting antimicrobial agent, which may fulfill the criteria described above (fig. 1). Chitosan is a natural carbohydrate polymer derived from the deacetylation of chitin. Chitosan have different degrees of deacetylation and molecular mass, and is produced commercially from crab and shrimp shell wastes. Because of chitosan’s promising biological activities, including non-toxicity and antimicrobial activity, it is used for a variety of purposes in food production, medicine, agriculture, cosmetics and biotechnology. The antimicrobial properties are thought to be due to positively charged amino groups, NH₃⁺, participating in an electrostatic interaction with negative charged groups on the cell surface of the bacteria. This may damage the cell wall and change its permeability and barrier properties allowing the cell contents to leak out. Chitosan has shown antimicrobial effect against oral bacteria and is tested for use as antimicrobial agent in composites and other dental materials and oral hygiene products.

Experiments with low viscosity chitosan have shown an antibacterial dose-response effect after being dissolved in culture media at various concentrations (fig. 2) and reduced biofilm when coated on polystyrene discs. We are currently investigating the use of chitosan in dental composites.

Reference:
The oral cavity is one of the human habitats with the highest diversity of bacterial species. It is estimated to be over 700 bacterial species that are capable of residing in this habitat. Bacteria were long thought of living as free floating, planktonic, cells. However, it has become clear that the preferred form of living is in a biofilm. Biofilm is a community of bacteria living on a surface, embedded in an extracellular matrix composed of proteins, polysaccharides and DNA. Oral plaque is a typical example of a multispecies biofilm. Development of a dental biofilm is an ordered sequence of events, starting with pioneer species attaching to the pellicle coating the surface. The attached bacteria will grow and modify the environment, this enables that more fastidious bacteria can colonize and join the biofilm. In the biofilm, different bacterial species are living in close proximity to one another, which enables both intra and inter-species interactions. The biofilm phenotype of a bacteria is distinct from the same species when grown planktonically. Biofilms have increased tolerance to antimicrobials, and provide a greater protection from the host defense, which makes them difficult to treat.

If changes occur in the environment, the proportion of the different bacteria in the biofilm may change. Such a change may occur with frequent intake of fermentable carbohydrates which selects for the growth of acid tolerant and acidogenic species in the biofilm. Such an ecological shift in the biofilm on a tooth, may lead to caries development.

To study oral biofilms in the laboratory, we can use different species separate (monospecies biofilm) or several species together (multispecies biofilm). NIOM have a special interested in studying different antibacterial compounds and their effect on the formation and growth of biofilm. Such compounds can be incorporated or coated on the surface of the biomaterial investigated. NIOM has a number of techniques available for studying biofilm properties. The amount of biofilm can be quantified after safranin staining (Fig. 1), and also be visualized by electron microscopy (Fig. 2). The viability of the biofilm can be determined by live/dead staining and fluorescence microscopy (Fig. 3) and by viability counting after detaching the biofilm followed by plating on nutrient agar plates.