Combating dental decay

Dental caries

Dental caries or tooth decay is one of the most prevalent bacterial infectious diseases of mankind. In recent oral health surveys, more than 60% of Australian teenagers surveyed had experienced the disease and most dentate adults surveyed exhibited multiple teeth affected by caries. Treating the consequences of dental caries accounts for over 50% of the total cost of providing dental services in Australia, which in 1998 was estimated at $2.6 billion. Dental caries is a dynamic process that is initiated by microbial biofilms on the tooth surfaces (dental plaque) resulting in a disturbance of the equilibrium between tooth mineral and the surrounding plaque fluid so that over time there is a net loss of mineral from the tooth surface. This demineralisation of the enamel may ultimately lead to cavitation of the surface of the tooth and once this stage of the disease has been reached only restorative methods (fillings) can be employed to limit the spread of decay and eventual loss of the tooth. Whilst many of the over 700 species of oral bacteria are saccharolytic and ferment dietary sugars to acidic end products few are able to produce acid at a sufficient rate to overcome salivary buffering capacity and rapidly lower plaque pH. Most species are also unable to continue metabolism in the acidic environment (approximately pH 5.2) that is necessary to cause enamel demineralisation and initiation of the caries process. The main bacterial species associated with caries initiation are members of the ‘mutans streptococcus group’ as these species are both strongly acidogenic and aciduric. Along with animal model testing, analysis of many clinical studies has indicated that Streptococcus mutans, Streptococcus sobrinus and Lactobacillus casei are the species most commonly associated with dental caries. These species are part of the normal oral microflora and are regarded as opportunistic pathogens. The application of new technologies such as transcriptomic and proteomic analyses will provide more detailed insights into the virulence factors of these species. Fluoride has been the main weapon in the arsenal against caries and is undoubtedly one of the most effective preventive therapies. However, its extensive use has failed to eliminate the disease and recent Australian and overseas survey data indicate that caries prevalence in the community is no longer decreasing and in some groups is increasing.

Novel preventive and treatment strategies

Much of the microbiological research into caries prevention is based on the belief that S. mutans is the major causative agent of caries initiation and development and that removal of this bacterium from dental plaque or modification of its virulence will stop or greatly reduce the incidence of dental caries in individuals – a still as yet untested hypothesis.

Replacement therapy

Jeff Hillman and colleagues at the University of Florida, Gainesville have developed a novel ‘replacement therapy’ where the indigenous strains of S. mutans are displaced by an avirulent strain. Convinced that the major virulence factor of S. mutans is its ability to produce lactic acid at a rapid rate, these researchers have genetically engineered a strain of S. mutans to produce ethanol instead of lactic acid. This was achieved by replacing the S. mutans lactate dehydrogenase gene with a Zymomonas mobilis ethanol dehydrogenase gene. In batch culture, the mutant produced less acid than the wild-type strain. However, its slower growth rate did not give it the competitive edge needed to displace the indigenous strains of S. mutans already present in the mouth. This competitive edge was provided by inserting a naturally occurring plasmid into the already modified ethanol-producing strain. The plasmid encoded a ‘lantibiotic’ (mutacin 1140) that is lethal to most other S. mutans strains. Animal testing indicated that this strain did displace other S. mutans strains and lead to a reduced level of caries, but concerns about the introduction of genetically modified organisms to the oral cavity have so far slowed clinical trials in humans. The technology is currently being pursued by Oragenics (http://www.oragenics.com) who have been refused permission to test this replacement therapy in Phase I human trials because of fears of horizontal transmission of the genetically modified bacterium. Recently, permission has been granted to undertake a Phase I safety trial using an auxotrophic strain and denture wearing participants to determine the level of...
transmission of the bacterium. Given the level of caution about testing a genetically modified bacterium it begs the question, will consumers be willing to allow a mouth rinse containing genetically modified bacteria to be administered to their children to slow the development of caries, even if the technology is shown to be effective?

Caries vaccine

The development of caries vaccines has been investigated since the late 1960s but has met with little success due to the location of the bacteria in fissures of the back teeth that are inaccessible to antibodies and the potential cross-reactivity of streptococcal antigens. The best results to date have been obtained by stimulating a salivary IgA response against S. mutans, which only resulted in a short term suppression of the bacterium in plaque. However, proponents of this strategy contend that improvements in vaccine delivery technology and the characterisation of species specific surface proteins will improve results.

Julian Ma, Tom Lehner and colleagues from Guy’s Hospital, London have developed a novel, passive vaccine against S. mutans. In an impressive piece of research, they purified the major S. mutans adhesin (SpaI/II) and used this to produce a monoclonal antibody from murine spleen cells. The genes encoding the monoclonal antibody were then inserted into a tobacco plant engineered to over-express the assembled antibody. This recombinant monoclonal antibody, or plantibody, binds specifically to S. mutans and interferes with its ability to colonise dental plaque. Prior to antibody application, antiseptic treatment is used to greatly reduce the levels of S. mutans and many other bacteria in plaque. The antibody interferes with adhesion of S. mutans to plaque whilst recolonisation by other oral bacterial species occurs unimpeded. This treatment is reported to ecologically remove S. mutans from the mouth for up to six months. Animal testing of the monoclonal antibody demonstrated a sustained reduction in S. mutans in plaque and a corresponding decrease in caries. This technology is now being commercialised by the Planet company as CaroRx™. This treatment has completed US FDA Phase I clinical trials and is currently undergoing Phase II clinical trials.

Anticariogenic peptides

At the CRC for Oral Health Science, we have taken a more biochemically-based approach to caries prevention by developing a technology that increases the concentration of calcium and phosphate, the major components of enamel, at the tooth surface. Using synthetic phosphopeptides we identified the motifs (Ser(P)-Ser(P)-Ser(P)-Glu-Glu-) within the bovine milk protein casein, responsible for stabilisation of amorphous calcium phosphate allowing milk to become supersaturated with bioavailable calcium and phosphate. By extracting these phosphopeptides we have produced a novel, bioavailable, amorphous form of calcium phosphate (ACP) stabilised by the casein phosphopeptides (CPP). The stabilised CPP-ACP form nanocomplexes and their 3D structure demonstrate the way in which the casein phosphopeptides act to stabilise a core of calcium and phosphate that maintains supersaturation (Figure 1). The CPP-ACP nanocomplexes substantially increase the levels of calcium and phosphate in dental plaque thereby depressing enamel demineralisation and facilitating remineralisation. Extensive testing in animal and in situ trials has demonstrated the ACP-CPP nanocomplexes to be anticariogenic. This technology has been patented and developed commercially and is available world-wide as the anticariogenic additive Recaldent™.

Antibacterial peptides

Continuing with the theme of investigating bioactive peptides derived from bovine milk we have discovered a novel antimicrobial peptide, Kappacin that has activity against S. mutans growing as a biofilm. In vivo, oral streptococci grow as part of a complex polymicrobial biofilm (supragingival dental plaque) adhering to the surface of the tooth, embedded in a matrix of bacterial and host polymers. Growth as a biofilm offers a number of significant advantages to the bacterium over planktonic growth not the least of which is transmission of the bacterium. Given the level of caution about testing a genetically modified bacterium it begs the question, will consumers be willing to allow a mouth rinse containing genetically modified bacteria to be administered to their children to slow the development of caries, even if the technology is shown to be effective?

Caries vaccine

The development of caries vaccines has been investigated since the late 1960s but has met with little success due to the location of the bacteria in fissures of the back teeth that are inaccessible to antibodies and the potential cross-reactivity of streptococcal antigens. The best results to date have been obtained by stimulating a salivary IgA response against S. mutans, which only resulted in a short term suppression of the bacterium in plaque. However, proponents of this strategy contend that improvements in vaccine delivery technology and the characterisation of species specific surface proteins will improve results.

Julian Ma, Tom Lehner and colleagues from Guy’s Hospital, London have developed a novel, passive vaccine against S. mutans. In an impressive piece of research, they purified the major S. mutans adhesin (SpaI/II) and used this to produce a monoclonal antibody from murine spleen cells. The genes encoding the monoclonal antibody were then inserted into a tobacco plant engineered to over-express the assembled antibody. This recombinant monoclonal antibody, or plantibody, binds specifically to S. mutans and interferes with its ability to colonise dental plaque. Prior to antibody application, antiseptic treatment is used to greatly reduce the levels of S. mutans and many other bacteria in plaque. The antibody interferes with adhesion of S. mutans to plaque whilst recolonisation by other oral bacterial species occurs unimpeded. This treatment is reported to ecologically remove S. mutans from the mouth for up to six months. Animal testing of the monoclonal antibody demonstrated a sustained reduction in S. mutans in plaque and a corresponding decrease in caries. This technology is now being commercialised by the Planet company as CaroRx™. This treatment has completed US FDA Phase I clinical trials and is currently undergoing Phase II clinical trials.

Anticariogenic peptides

At the CRC for Oral Health Science, we have taken a more biochemically-based approach to caries prevention by developing a technology that increases the concentration of calcium and phosphate, the major components of enamel, at the tooth surface. Using synthetic phosphopeptides we identified the motifs (Ser(P)-Ser(P)-Ser(P)-Glu-Glu-) within the bovine milk protein casein, responsible for stabilisation of amorphous calcium phosphate allowing milk to become supersaturated with bioavailable calcium and phosphate. By extracting these phosphopeptides we have produced a novel, bioavailable, amorphous form of calcium phosphate (ACP) stabilised by the casein phosphopeptides (CPP). The stabilised CPP-ACP form nanocomplexes and their 3D structure demonstrate the way in which the casein phosphopeptides act to stabilise a core of calcium and phosphate that maintains supersaturation (Figure 1). The CPP-ACP nanocomplexes substantially increase the levels of calcium and phosphate in dental plaque thereby depressing enamel demineralisation and facilitating remineralisation. Extensive testing in animal and in situ trials has demonstrated the ACP-CPP nanocomplexes to be anticariogenic. This technology has been patented and developed commercially and is available world-wide as the anticariogenic additive Recaldent™.

Antibacterial peptides

Continuing with the theme of investigating bioactive peptides derived from bovine milk we have discovered a novel antimicrobial peptide, Kappacin that has activity against S. mutans growing as a biofilm. In vivo, oral streptococci grow as part of a complex polymicrobial biofilm (supragingival dental plaque) adhering to the surface of the tooth, embedded in a matrix of bacterial and host polymers. Growth as a biofilm offers a number of significant advantages to the bacterium over planktonic growth not the least of which is transmission of the bacterium. Given the level of caution about testing a genetically modified bacterium it begs the question, will consumers be willing to allow a mouth rinse containing genetically modified bacteria to be administered to their children to slow the development of caries, even if the technology is shown to be effective?

Caries vaccine

The development of caries vaccines has been investigated since the late 1960s but has met with little success due to the location of the bacteria in fissures of the back teeth that are inaccessible to antibodies and the potential cross-reactivity of streptococcal antigens. The best results to date have been obtained by stimulating a salivary IgA response against S. mutans, which only resulted in a short term suppression of the bacterium in plaque. However, proponents of this strategy contend that improvements in vaccine delivery technology and the characterisation of species specific surface proteins will improve results.

Julian Ma, Tom Lehner and colleagues from Guy’s Hospital, London have developed a novel, passive vaccine against S. mutans. In an impressive piece of research, they purified the major S. mutans adhesin (SpaI/II) and used this to produce a monoclonal antibody from murine spleen cells. The genes encoding the monoclonal antibody were then inserted into a tobacco plant engineered to over-express the assembled antibody. This recombinant monoclonal antibody, or plantibody, binds specifically to S. mutans and interferes with its ability to colonise dental plaque. Prior to antibody application, antiseptic treatment is used to greatly reduce the levels of S. mutans and many other bacteria in plaque. The antibody interferes with adhesion of S. mutans to plaque whilst recolonisation by other oral bacterial species occurs unimpeded. This treatment is reported to ecologically remove S. mutans from the mouth for up to six months. Animal testing of the monoclonal antibody demonstrated a sustained reduction in S. mutans in plaque and a corresponding decrease in caries. This technology is now being commercialised by the Planet company as CaroRx™. This treatment has completed US FDA Phase I clinical trials and is currently undergoing Phase II clinical trials.

Anticariogenic peptides

At the CRC for Oral Health Science, we have taken a more biochemically-based approach to caries prevention by developing a technology that increases the concentration of calcium and phosphate, the major components of enamel, at the tooth surface. Using synthetic phosphopeptides we identified the motifs (Ser(P)-Ser(P)-Ser(P)-Glu-Glu-) within the bovine milk protein casein, responsible for stabilisation of amorphous calcium phosphate allowing milk to become supersaturated with bioavailable calcium and phosphate. By extracting these phosphopeptides we have produced a novel, bioavailable, amorphous form of calcium phosphate (ACP) stabilised by the casein phosphopeptides (CPP). The stabilised CPP-ACP form nanocomplexes and their 3D structure demonstrate the way in which the casein phosphopeptides act to stabilise a core of calcium and phosphate that maintains supersaturation (Figure 1). The CPP-ACP nanocomplexes substantially increase the levels of calcium and phosphate in dental plaque thereby depressing enamel demineralisation and facilitating remineralisation. Extensive testing in animal and in situ trials has demonstrated the ACP-CPP nanocomplexes to be anticariogenic. This technology has been patented and developed commercially and is available world-wide as the anticariogenic additive Recaldent™.

Antibacterial peptides

Continuing with the theme of investigating bioactive peptides derived from bovine milk we have discovered a novel antimicrobial peptide, Kappacin that has activity against S. mutans growing as a biofilm. In vivo, oral streptococci grow as part of a complex polymicrobial biofilm (supragingival dental plaque) adhering to the surface of the tooth, embedded in a matrix of bacterial and host polymers. Growth as a biofilm offers a number of significant advantages to the bacterium over planktonic growth not the least of which is transmission of the bacterium. Given the level of caution about testing a genetically modified bacterium it begs the question, will consumers be willing to allow a mouth rinse containing genetically modified bacteria to be administered to their children to slow the development of caries, even if the technology is shown to be effective?
is protection against antimicrobial agents. We have developed methods for the culture of S. mutans and other oral bacteria as biofilms in a constant depth film fermenter (CDFF). This device provides a sophisticated means of reproducing large numbers of biofilms that can be used for antimicrobial testing. Unlike testing against planktonic bacteria, results obtained with the CDFF have a predictive value on the efficacy of the antimicrobial agent in the oral cavity. An antimicrobial peptide that is able to decrease S. mutans levels in plaque has potential in combating caries.

New opportunities
The growth of oral streptococci as part of a polymicrobial biofilm also offers the opportunity to interfere with the intercellular signalling systems that many of these species use to communicate and regulate gene expression. Increasingly, the quorum-sensing peptides of these bacteria look to hold promise for manipulating the composition of oral biofilms and, possibly, modulating their virulence.15

Conclusion
The only effective anticaries agent that has been developed in the last fifty years is fluoride. However, recent research has resulted in the development of other agents (specific antibodies, CPP-ACP and antibacterial peptides) that show promise in supplementing the action of fluoride to help prevent dental caries.

References

ams Laboratories are offering food and pharmaceutical manufacturers an advanced bacterial identification and characterisation service using RiboPrinter® system technology. This leading edge system offers tangible improvements in quality control that ultimately deliver greater consumer confidence to brand users.

BENEFITS
• Distinguish different isolates of the same species and thereby trace the origins of product contamination.
• Customized database libraries created by ams Laboratories enable re-occurring contaminants to be pinpointed below species level.
• Your database grows with your sampling regimen.

For further Information:
Paul Priscott PhD
Managing Director
ams Laboratories Pty Ltd
Phone: 02 9567 8544
Fax: 02 9567 8228
Email: info@amslabs.com.au

RiboPrinter® is a US-registered trademark of Qualcon Inc., Wilmington, Delaware.