Clinical

The biochemical basis of dental caries in action

Jemma Gewargis discusses the fundamentals of aiming to remineralise carious lesions at an early stage to avoid further tooth damage

Dental caries and the carious process

Dental caries is essentially a dynamic process involving a microbial deposit, the dental biofilm on the tooth surface, which undergoes several metabolic reactions, resulting in chemical dissolution of the tooth substance (Fejerskov and Kidd, 2013).

The dental biofilm is a community of metabolically active microorganisms that have adhered to the tooth surface (Kidd, 2005). Bacteria within the biofilm metabolise fermentable carbohydrates from the diet, and organic acids are produced, which dissolve the hydroxyapatite component of teeth (Caufield and Griffen, 2000). The acidic environment selects for the microorganisms best able to withstand this low pH, therefore these organisms flourish and continue the carious process.

It is the frequency of carbohydrate consumption that plays such a pivotal role in the carious process. Patients who repeatedly ingest high levels of carbohydrates have recurring drops in the salivary pH as the buffering capacity is overwhelmed (Wade, 2013).

The Critical pH is a key biochemical value in the carious process and is 5.5 for enamel, indicating the highest pH at which there is net mineral loss of tissue from the tooth, ie, the pH at which demineralisation begins. Dentine is composed of more organic material and water, meaning it is more easily degraded in the carious process and so, the critical pH is higher (more alkaline) at approximately 6.2 (Masthan et al, 2013).

Biochemistry of plaque

The dental plaque biofilm can be defined as a 'three-dimensional structure consisting of densely packed aggregations of microbes' that adhere to the each other and the tooth surface (Mancl et al, 2013). Attachment of the pioneer species is the earliest phase of biofilm formation. This is subsequently followed by succession of other colonising species, which modify the oral environment to favour the development of a successful climax community (Mancl, 2013).

Streptococcus mutans is recognised as the principal species in caries due to its property of aciduricity, allowing it to tolerate acidic environments. Thus, during the carious process, streptococcus mutans is able to thrive and multiply as a result of its high tolerance to the low pH environment (Burne et al, 2012).

Streptococcus mutans derived glucosyltransferases (Gtfs) are important in the process of plaque development. Studies have concluded that specific Gtfs were upregulated in response to a more acidic environment, aiding colonisation by other bacteria and the cohesion of plaque, contributing to a more caries-susceptible environment (Bowen et al, 2011; Thomas et al, 2012).

As plaque matures and the microbiota adapts, there is a change from a streptococcus-dominant to an actinomyces-dominant bacterial community (Takahashi and Nyvad, 2011).

Biochemical role of carbohydrates in caries

The concentration of the non-milk extrinsic sugars (NMES) and sucrose, in particular, play the most significant contribution to dental caries (Masson et al, 2010). Sucrose is considered to be the dietary carbohydrate that has the highest cariogenic potential with its effects being both frequency and concentration-dependent (Ccahuana-Vasquez et al, 2007).

The Maillard reaction involves the biochemical interactions between sugars and proteins that are responsible for the lesion discolouration in the carious process. Otherwise known as the 'Browning reaction' (Dyer, 1991), it is categorised as a non-enzymatic/glycation process (Kleter, 1998). It involves an irreversible change in the amino acid structures that make up proteins, hence altering their function. The reactions that proceed lead to the formation of brown polymers called melanoidins (Kleter, 1998), which are responsible for the colour change. The Maillard reaction has important consequences for clinicians as it has been discovered that the extent of disease can be interpreted from the severity of brown discolouration.

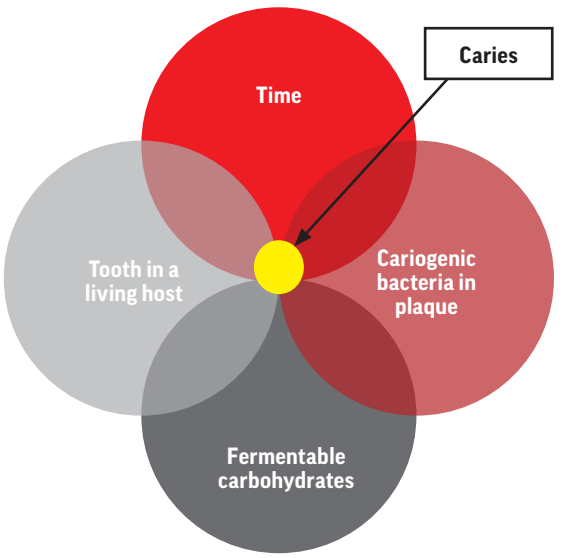


Figure 1: The aetiology of caries

Biochemistry of demineralisation

Demineralisation begins with increased enamel porosity, which can lead to cavitation and eventually tooth loss if untreated.

Organic acid production (in the form of H+) as a result of bacteria digesting sugars causes the pH of plaque to fall, resulting in dissolution of HA into calcium ions, hydrogen phosphate ions and water, which begins the process of demineralisation within enamel (Ilie et al, 2012).

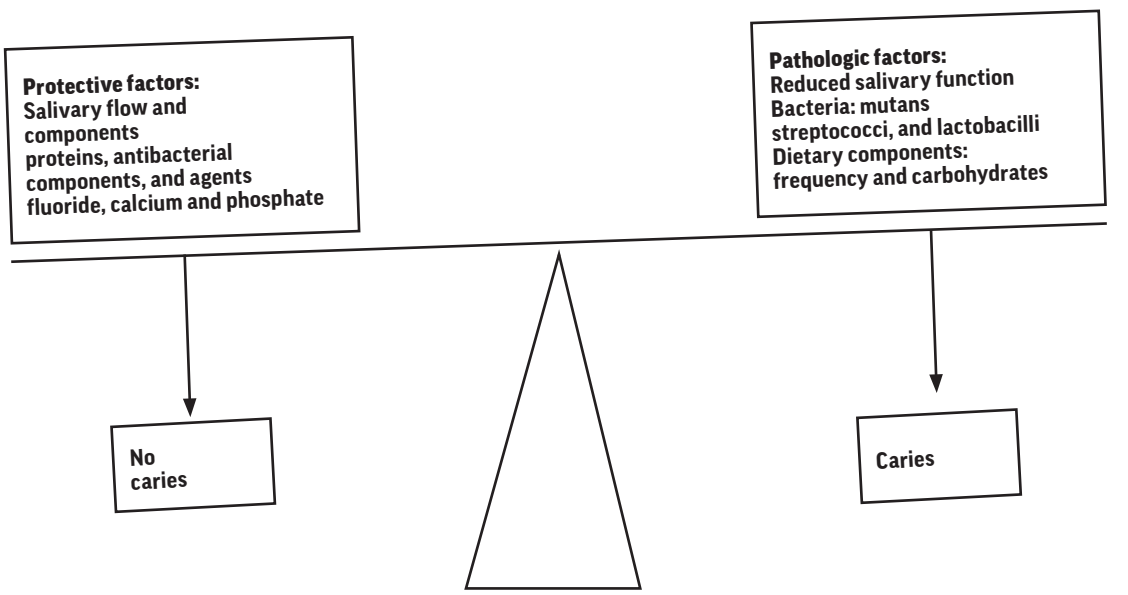
Mineral loss from the enamel which leaches into the surrounding saliva and plaque is ultimately the cause of white spot lesion (WSL) formation (Greene and Bearn, 2013). The mineral is replaced by water, which reduces the refractive index and so light cannot enter the enamel rods as far before being scattered back towards the surface (Ilie et al, 2012). This reflection is responsible for the appearance of the WSL.

Upon remineralisation, a dense layer of calcium, phosphate and fluoride forms on the surface that has greater resistance to further demineralisation (Featherstone, 2008). If demineralisation persists, a point is reached where the enamel subsurface has been so reduced that the outer enamel surface cannot support or withstand any biting forces, and so cavitation results (Greene and Bearn, 2013).

Research has lead to the discovery of an innovative type of remineralisation technology with the potential to improve or even completely restore the appearance of a WSL. Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) is based upon phosphopeptides from milk casein (Mount, 2012). Its unique ability to penetrate into the enamel porosities and stabilise calcium phosphate allow it to remineralise tooth tissue, eliminating the porous effect and restoring the light properties and translucency to improve the appearance of the WSL (Greene and Bearn, 2013; Mount, 2012; Kitasako et al, 2010).

Demineralisation of dentine results when the critical pH of 6.2 is reached, causing the dissolution of mineral (Zavgorodniy et al, 2008). Two dentine layers form, described as an outer (infected) layer and inner (affected) layer. Within the inner carious dentine, the collage fibrils maintain their shape (and thus can be remineralised) but the collagen within the outer carious dentine (closest to the coronal tooth surface) changes shape due to its degradation (Nakornchai et al, 2004). It is the ability

Figure 2: The carious process – the balance between the pathologic and protective factors (Touger-Decker and Van Loveren, 2003)2013)



Clinical

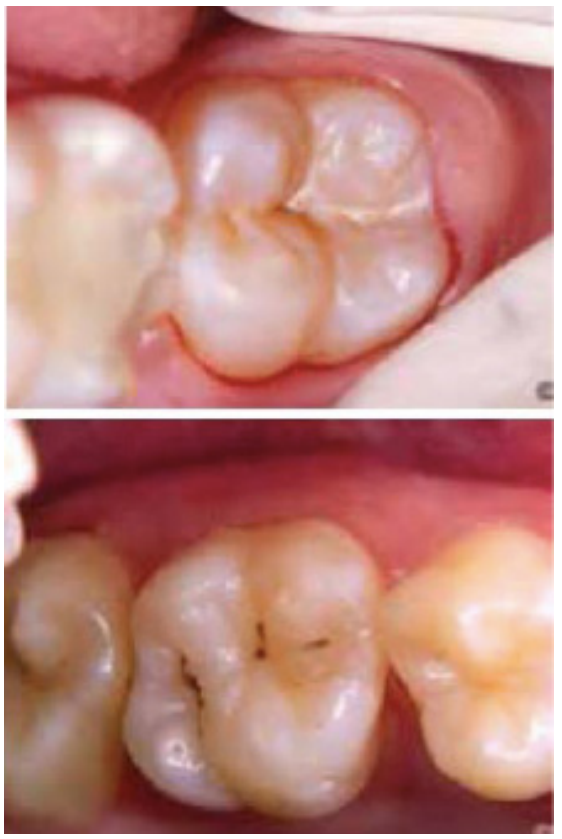


Figure 3: The carious lesion – occlusal, non-cavitated carious lesions with some staining (Fejerskov and Kidd, 2013)

to distinguish between the layers during carious dentine removal that is significant in our clinical practice.

In response to carious attack, the dentine acts to defend itself and the pulp by exhibiting different reactionary mechanisms such as tubular sclerosis – a reaction that remineralises the tubules with 'Mg-substituted β-TCP (whitlockite)' and apatite crystals (Zavgorodniy et al, 2008).

The biochemistry of saliva in relation to caries

It is the hydrogen bicarbonate balance in saliva that is responsible for the buffering capacity and pH, which acts to protect the enamel from caries (Cunha-Cruz et al, 2013).

Saliva is maintained at neutral pH by the action of buffers including inorganic phosphate in resting saliva and the carbonic acid-bicarbonate system in stimulated saliva, the latter of which is recognised as the major salivary buffer (Cunha-Cruz et al, 2013).

An increased salivary flow influences the biochemistry of the carious process as it results in a rise in the concentration of bicarbonate, chloride and sodium (Cunha-Cruz et al, 2013). The elevated bicarbonate concentration neutralises the acids, promoting the remineralisation of tooth tissue (Cunha-Cruz et al, 2013). Thus, ensuring our patients have adequate salivary flow is an important contributor to our clinical prevention of caries.

The enzymes involved in the carious process

Matrix metalloproteinases (MMPs) are categorised as zinc-dependent, host-derived proteolytic enzymes, which are important in the degradation of the organic matrix of dentine in the carious process (Masthan et al, 2013; Varun et al, 2012).

Research has found that pH changes in the carious lesion activate MMPs (Varun et al, 2012). MMPs only function at neutral pH and so the salivary buffer systems neutralise the acidic pH, allowing the MMPs to become activated to degrade the organic matrix of dentine (Chaussain-Miller et al, 2006).

The activation of MMPs is also dependent on the presence of zinc. However, interestingly, it has been reported that as well as this, zinc can influence the signalling pathway of MMPs and result in dentine remineralisation (Toledano et al, 2012). This makes zinc an attractive element to exploit clinically as a therapeutic agent for the remineralisation of tooth tissue. Zinc-leaching dental materials such as amalgams, zinc phosphate cement, calcium hydroxide and zinc-oxide eugenol cements are thought to inhibit the bacterial growth. The anion adheres to the enamel, forming Fluorohydroxyapatite, which is more resistant to acid attack. The antimicrobial property of fluoride ions involves inhibition of the enzyme Enolase in the Embden-Meyerhof-Parnas pathway, and so the bacteria are no longer able to thrive and promote tooth demineralisation in the carious process (Zero et al, 2009).

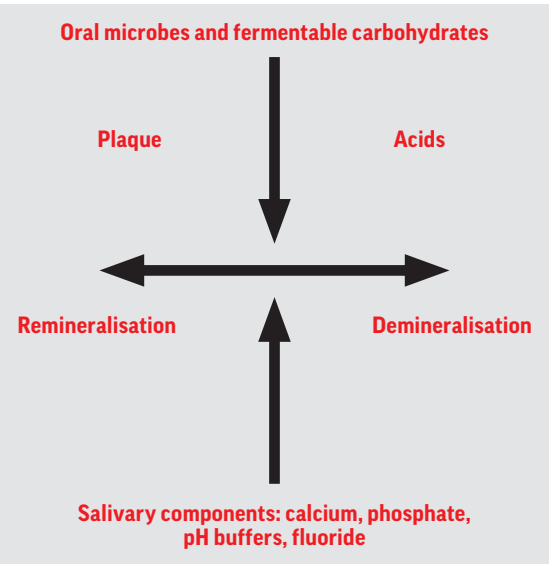


Figure 4: The demineralisation-remineralisation process in oral plaque – a summary of the influence of the saliva on the demineralisation-remineralisation balance (Stookey, 2008)2013)

demineralisation of dentine and promote remineralisation (Chaussain-Millar, 2006; Toledano et al, 2012).

The biochemistry of remineralisation and caries prevention

Xylitol is now widely recognised as having anticariogenic properties when used as a sweetener. Additionally, cranberries can reduce the glucosyltransferase activity of Streptococcus mutans, decreasing bacterial adherence (Zero et al, 2009). Furthermore, tea extracts are thought to inhibit salivary amylase activity, reducing the presence of fermentable carbohydrates in the oral cavity (Zero et al, 2009).

It is well established that the fluoride anion can inhibit

Conclusion

In conclusion, the scientific knowledge behind caries forms the basis of the decisions made by clinicians on a regular basis, including the extent of caries removal. It also governs the chemical concepts behind anticariogenic agents and other therapeutic means in the treatment and prevention of caries. The knowledge of the biochemistry of dental caries is vital, and always will be, to every clinician and will continuously be applied for generations to come. D

References

Bowen WH, Koo H (2011) Biology of Streptococcus Mutans-derived Glucosyltransferases: Role in Extracellular Matrix Formation of Cariogenic Biofilms. Caries research **45(1):** 69-86.

Breaker RR (2012) New Insight on the Response of Bacteria to Fluoride. Caries research **46(1):** 78-81.

Burne RA, Zeng L, Ahn SJ, Palmer SR, Liu Y, Lefebure T, Stanhope MJ, Nascimento MM (2012) Progress Dissecting the Oral Microbiome in Caries and Health. Advances in dental research **24(2):** 77-80.

Caufield PW, Griffen AL (2000) Dental caries. An infectious and transmissible disease. Paediatric Clinics of North America **47(5):** 1001-1019.

Ccahuana-Vasquez RA, Tabchoury CP, Tenuta LM, Del Bel Cury AA, Vale GC, Cury JA (2007) Effect of Frequency of Sucrose Exposure on Dental Biofilm Composition and Enamel Demineralization in the Presence of Fluoride. Caries research **41(1):** 9-15.

Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S (2006) The Role of Matrix Metalloproteinases (mmps) in Human Caries. Journal of dental research **85(1):** 22-32.

Cunha-Cruz J, Scott J, Rothen M, Mancl L, Lawhorn T, Brossel K, Berg J, Northwest Practice-based Research Collaborative in Evidence-Based Dentistry (2013) Salivary Characteristics and Dental Caries: Evidence from General Gental Practices. Journal of the american dental association **144(5):** 31-40.

Dyer DG, Blackledge JA, Katz BM, Hull CJ, Adkisson HD, Thorpe SR, Lyons TJ, Baynes JW (1991) The Maillard Reaction in Vivo. Zeitschrift fur ernahrungswissenschaft **30(1):** 29-45.

Featherstone JD (2008) Dental Caries: A Dynamic Disease Process. Australian dental journal **53(3):** 286-291.

Fejerskov O, Kidd E (2013) Changing Concepts in Cariology: Forty Years on. Dental Update **40:** 277-286.

Greene LE, Bearn DR (2013) Reducing White Spot Lesion Incidence During Fixed Appliance Therapy. Dental update **40(6):** 487-490.

Iiie O, Van Loosdrecht MC, Picioreanu C (2012) Mathematical Modelling of Tooth Demineralisation and ph Profiles in Dental Plaque. Journal of theoretical biology **309:** 159-175.

Kidd E (2005) Essentials of dental caries: the disease and its management 3rd edition. Oxford: Oxford University Press.

Kitasako Y, Cochrane NJ, Khairul M, Shida K, Adams GG, Burrow MF, Reynolds EC, Tagami J (2010) The Clinical Application of Surface ph Measurements to Longitudinally Assess White Spot Enamel Lesions. Journal of dentistry **38(7):** 584-590.

Kleter GA (1998) Discoloration of Dental Carious Lesions (A Review). Archives of oral biology **43(8):** 629-632.

Mancl KA, Kirsner RS, Ajdic D (2013) Wound Biofilms: Lessons Learned from Oral Biofilms. Wound repair and regeneration **21:** 352-362.

Masson LF, Blackburn A, Sheehy C, Craig LC, Macdiarmid JI, Holmes BA, Mcneill G (2010) Sugar Intake and Dental Decay: Results from a National Survey of Children in Scotland. British journal of nutrition **104(10):** 1555-1564.

Masthan K, Babu N, Bhattacharjee T, Elumalai M (2013) Biochemical Markers – A Tool to Detect Oral Diseases. International journal of pharma and bio sciences **4(1):** 819-827.

Mount G (2012) Minimal Intervention in Dentistry: Glass-ionomers, Composite Resins and Cpp-acp. Journal of minimal intervention in dentistry **5:** 186-9.

Nakornchai S, Atsawasuwan P, Kitamura E, Surarit R, Yamauchi M (2004) Partial Biochemical Characterisation of Collagen in Carious Dentin of Human Primary Teeth. Archives of oral biology **49(4):** 267-273.

Takahashi N, Nyvad B (2011) The Role of Bacteria in the Caries Process: Ecological Perspectives. Journal of dental research **90(3):** 294-303.

Thomas RZ, Zijnge V, Cicek A, De Soet JJ, Harmsen HJ, Huysmans MC (2012) Shifts in the Microbial Population in Relation to in situ Caries Progression. Caries research **46(5):** 427-431.

Toledano M, Yamauti M, Osorio E, Osorio R (2012) Zinc-inhibited Mmp-mediated Collagen Degradation After Different Dentine Demineralization Procedures. Caries research **46(3):** 201-207.

Varun BR, Nair, Bindu J, Sivakumar TT, Joseph, Anna P (2012) Matrix metalloproteinases and their role in oral diseases: a review. Oral & maxillofacial pathology journal **3(1):** 186-191.

Wade WG (2013) The oral microbiome in health and disease. Pharmacological Research **69(1):** 137-143.

Zavgorodniy AV, Rohanizadeh R, Swain MV (2008) Ultrastructure of Dentine Carious Lesions. Archives of oral biology **53(2):** 124-132.

Zero DT, Fontana M, Martinez-Mier EA, Ferreira-Zandona A, Ando M, Gonzalez-Cabezas C. Bayne S (2009) The Biology, Prevention, Diagnosis and Treatment of Dental Caries: Scientific Advances in the United States. Journal of the american dental association **140(suppl 1):** 25s-34s.

Having recently graduated from KCL Dental Institute, Jemma is currently enjoying her DFT1 post within the Berkshire Scheme. She possesses a keen interest in caries and restorative dentistry where additional reading has allowed further development in her knowledge of these topics. Jemma is looking forward to applying these principles at an early start of her clinical career with an aim to provide the highest levels of patient care.