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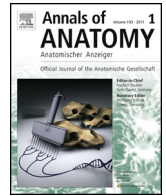
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Research Article

# The 'sialo–microbial–dental complex' in oral health and disease

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## ABSTRACT

Biofilms are naturally found in all wet environments including the oral structures of nearly all species. Human oral biofilms have existed since our earliest ancestors and have evolved symbiotically with the dentition over many millennia within a Palaeolithic, hunter–gatherer setting. Irrespective of the plant–animal ratio, it can be argued that the Palaeolithic diet was essentially acidic, and acted as a selective force for much of the evolution of the stomatognathic system. The relationship between saliva, biofilm and teeth, the 'sialo–microbial–dental complex', provides oral health benefits and offers a different perspective to the old dental paradigm that only associated oral biofilms (plaque) with disease (caries). This new paradigm emphasises that oral biofilms are essential for the 'mineral maintenance' of teeth.

Oral biofilms provide physical protection from dietary acid and together with bacterial metabolic acids cause the resting pH of the biofilm to fall below neutral. This is then followed by the re-establishment of a neutral environment by chemical interactions mediated by the saliva within the biofilm. Such pH fluctuations are often responsible for the cyclic demineralisation, then remineralisation of teeth, a process necessary for tooth maturation.

However, since the advent of farming and especially since the industrial revolution, the increase in consumption of carbohydrates, refined sugars and acidic drinks has changed the ecology of biofilms. Biofilm biodiversity is significantly reduced together with a proliferation of acidogenic and aciduric organisms, tipping the balance of the 'demin–remin' cycle towards net mineral loss and hence caries. In addition, the consumption of acidic drinks in today's societies has removed the protective nature of the biofilm, leading to erosion.

Erosion and caries are 'modern-day' diseases and reflect an imbalance within the oral biofilm resulting in the demineralisation of teeth.

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## 1. Introduction

Within the anthropological literature, there is a common theme that is evident among Palaeolithic, hunter–gatherer populations. Dentitions showed extensive wear and the prevalence of oral diseases such as dental caries and periodontal diseases was very low, in contrast to contemporary populations (Campbell, 1925; Barrett, 1977). Even dental erosion that is so prevalent in contemporary populations was not apparent in Palaeolithic populations (Bell et al., 1998).

However, since farming practices began to evolve some 10,000 years ago, there began to be an increase in carbohydrate consumption leading to an unprecedented consumption of refined sugar since the industrial revolution. In addition, food preservation and fermentation techniques significantly increased the consumption of dietary acids, culminating in an excessive consumption

of acidic drinks within our modern culture. This monumental change in the human diet, was a 'sudden' environmental stress from an evolutionary perspective (Sebastian et al., 2002) that has had an impact on the oral ecosystem leading to caries, erosion and periodontal disease (Adler et al., 2013). These sudden lifestyle changes have not only been responsible for tipping the balance towards 'modern' oral diseases but they are also responsible for other systemic, non-communicable diseases (e.g., diabetes) that afflict our populations today (Hujöel, 2009; Ströhle et al., 2010).

This dichotomy in oral health between past and present populations has been documented at length over the years and the relationship between refined carbohydrates, oral biofilms and caries has been confirmed. Past paradigms have suggested that all biofilm (plaque) caused disease, however the current evidence indicates that the make-up of the biofilm determines its cariogenicity and can be explained by the 'the ecological plaque hypothesis' (Marsh, 1994). Nevertheless the perception that biofilm is a pathological entity still persists among researchers and clinicians and it is this mindset that needs to change!

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It can be argued that oral biofilms have co-evolved symbiotically with humans, representing over two million years of evolution within a hunter–gatherer (i.e., Palaeolithic) lifestyle, where the interplay between genes and differing environments has resulted in human and microbial adaptations responsible for maintaining good oral health. The human oral environment in health should therefore include not only all hard and soft tissues and salivary secretions, but also the oral microbiome. After all, this perspective is no different to our understanding of the microbiome of the human gut and how it is necessary for survival. Based on this premise, the oral biofilm has a physiological purpose that has been overlooked and that protects the teeth from dietary acids.

This paper proposes an ‘evolutionary model’ that represents a paradigm shift in thinking about how the oral environment evolved and how today’s lifestyle has impacted on a well-established ‘balanced’ system that has been in existence for over two million years. Aspects of this model are a synthesis of current yet disparate knowledge that also supports prevailing theories about oral disease. Although further research is required to confirm or refute aspects of the model, it promises to alter the way that clinicians view the oral environment and, in particular, patient management.

This ‘Evolutionary Model of Mineral Maintenance’ proposes that:

- The oral structures have always been exposed to dietary acids since the earliest of times.
- Dietary acids have been one of the selective forces that have influenced the evolution of the oral environment of hunter–gatherer populations for thousands of years.
- Oral biofilms and saliva have evolved a fundamental protective role against demineralisation resulting mainly from dietary acids.
- This paradigm shift supports current concepts of oral disease indicating that caries and erosion reflect an imbalance within the oral system.

## 2. The Palaeolithic diet and oral adaptations

Anthropologists have documented how ancestral hunter–gatherer human populations survived in varied and extreme environments, both spatially and temporally. As a result, diets varied in consistency, abrasiveness, hardness, pH and nutritional value and the foods available were often seasonal.

The impact of diet on the evolution of the human stomatognathic system, particularly the abrasivity of food, has resulted in evolutionary adaptations that have affected craniofacial morphology, tooth size and the chemical composition of the dental tissues (Kaidonis et al., 2012). For example, it is clear that enamel and dentine, both individually and in combination, have evolved over many millennia to withstand masticatory forces. The enamel resists wear and is structured in the form of inorganic enamel rods consisting of hydroxyapatite crystals forming one of the strongest biological materials. In addition, dentine supports the enamel thereby overcoming its brittle nature. However, when the enamel wears and dentine is exposed, the dentine wears faster. This differential wear in turn maintains masticatory efficiency (Every and Kuhne, 1971; Kaidonis et al., 1992).

The pH of food also exerted an evolutionary impact on the stomatognathic system and, though much is known about the pH of food and how it is metabolised by the body to regulate plasma pH levels, the pH of food while in the mouth and during mastication, has resulted in oral evolutionary adaptations that have not been documented within a cohesive model. The pH of food has been one of the selective forces responsible for many oral adaptations. For example, the most sensitive taste buds on the tongue are those that identify alkalis, representing an adaptive

response for protection against poisons (alkaloids). These taste buds are situated on the posterior (dorsal) surface of the tongue that is innervated by the glossopharyngeal nerve that in turn is responsible for the gagging and vomiting reflex, thereby adding to the protection. Alternatively, dietary acids within the mouth (e.g., lemon juice on the tongue) cause the parotid gland to produce copious amounts of stimulated saliva with high concentrations of bicarbonate ( $\text{HCO}_3^-$ ) ions. This saliva has a very high buffering capacity, an ability to very effectively neutralise acids.

Often anthropologists refer to the plant–animal ratio of the diet for any given population in order to obtain some idea of the existing variation between diets. This ratio can vary dramatically and has a bearing on the subsistence nutrient intake and therefore energy levels within and between populations (Cordain et al., 2000). Extreme variations have been recorded between populations. One example is the high protein diet consumed by the Inuit that survive on fish and animal products for the majority of the year, versus other populations living in lush environments that consume a higher proportion of plant material. However, irrespective of the plant–animal ratio, the vast majority of food (including meat) consumed by hunter–gatherers is acidic and although some alkalis were a part of the diet of Homo, the majority of alkalis are poisons (i.e., alkaloids). Further to this, except for the consumption of milk during breastfeeding, hunter–gatherers consumed mainly water when they were thirsty. This is in contrast to the high consumption of acidic drinks occurring in contemporary populations.

Plants have an extreme variation in pH, depending on the species, the component in question (i.e., flower, plant body or fruit) and in particular the season. Although, in general, the level of acidity of plants can range from mild to extreme, it must be remembered that continuous exposure (i.e., continuous ‘grazing’) has the potential of producing an accumulative erosive effect on teeth (i.e., demineralisation). However, erosion from skeletal material of hunter–gatherer populations is scarcely evident indicating that its prevalence was so low that it was insignificant. This is quite remarkable considering teeth are inorganic structures and can readily dissolve in acid. The answer to this dilemma lies in the physiological function of the biofilm that exists at the interface between the tooth and the acidic environment. The co-evolution of the biofilm with the dental tissues has resulted in a symbiotic relationship between the biofilm and the human host.

## 3. Sialo–microbial–dental complex

The vast majority of bacteria that exist in nature (99%) are found attached to wet or damp surfaces in the form of biofilms and include the wet oral structures of nearly all species, including Homo. The biodiversity of human oral biofilms in health is large (over 700 different species) and consists of many species living symbiotically with one another and with the human host. In addition, the biofilm acts as a semi-permeable membrane to nutrients, excreted bi-products and to various molecules allowing for inter and intracellular interactions. In fact, within the salivary rich biofilm there are many gradients (e.g., pH gradients, oxygen gradients, nutrient gradients etc.) that are responsible for how the different bacterial species arrange themselves with respect to one another, determining the ultimate final structure of the biofilm.

The biodiversity of a healthy oral biofilm within hunter–gatherers was large (Adler et al., 2013) and the pH of biofilms is generally neutral. More specifically, populations consuming more meat tend to have a resting biofilm pH of just over 7 (approx. pH 7.2) while those who consume more plant material will tend to have a resting pH that is slightly acidic (approx. pH 6.8). Many of the bacterial species within the biofilm prefer near neutral to alkaline environments and perpetuate this by metabolising urea (excreted

in saliva) and arginine (a salivary protein) to produce ammonia (alkali) and CO<sub>2</sub>. Species such as mutans streptococci that are acidogenic and aciduric (i.e., produce acid and prefer acidic conditions) make up approximately 2% of the biofilm species and therefore, on balance, are ineffective in causing demineralisation and dental caries.

It is not our intention to detail all of the interactions that occur within biofilms or elaborate on all of the functions of saliva. However, among the many functions of saliva is its ability to act as an ion reservoir (e.g., Ca<sup>++</sup>, PO<sub>4</sub><sup>3-</sup>, OH<sup>-</sup>, HCO<sub>3</sub><sup>-</sup> etc.) capable of neutralising acids and providing the building blocks for the remineralisation of hydroxyapatite. As a general rule these ions are very reactive and have the potential to precipitate out of solution, however certain salivary proteins (e.g., proline-rich proteins, statherins, etc.) ‘loosely’ bind or ‘stabilise’ such ions (e.g., Ca<sup>++</sup>) preventing them from reacting. This simple mechanism also prevents salivary stones from forming in gland ducts, a great evolutionary strategy.

The vegetarian component of the hunter-gatherer diet was considerably varied but did contain a high proportion of complex carbohydrates (e.g., tubers, etc.) and some simple sugars (e.g., fruit). The mastication of food would cause an acidification of the biofilm causing a drop in its resting pH, mainly from the acid nature of the food but also, to a smaller degree, from some bacterial metabolism. This was of no consequence to dental structures because of a number of evolved mechanisms. For example, a significant amount of acid is neutralised or buffered, initially by the PO<sub>4</sub><sup>3-</sup> ions in resting saliva, followed by the more effective HCO<sub>3</sub><sup>-</sup> ions from stimulated saliva.

However, when acids are not completely buffered and unsaturated conditions develop at the tooth-biofilm interface, demineralization of the tooth structure occurs. The Ca<sup>++</sup>, PO<sub>4</sub><sup>3-</sup> and OH<sup>-</sup> ions resulting from the dissolution of the enamel accumulate at the interface between the biofilm and tooth and, as the pH increases, supersaturated conditions slowly become restored. In addition, the initial drop in pH within the biofilm also has the simultaneous effect on salivary proteins (e.g., statherins and proline-rich proteins) causing them to release the bound Ca<sup>++</sup> and PO<sub>4</sub><sup>3-</sup> ions that had originated from saliva, thereby adding to the supersaturated conditions. As a result remineralisation occurs. This is ‘closed

system’ where the essential ions required for remineralisation are not allowed to escape because of the presence of the biofilm – an evolutionary strategy for ‘mineral maintenance’.

Furthermore, this natural demineralisation–remineralisation process is responsible for the maturation of newly emerged teeth. Repeated cycles over time cause the tooth to become less soluble to acid by progressively replacing the more soluble carbonate substituted apatite (found in young individuals) to less soluble hydroxyapatite and fluoroapatite when fluoride is present.

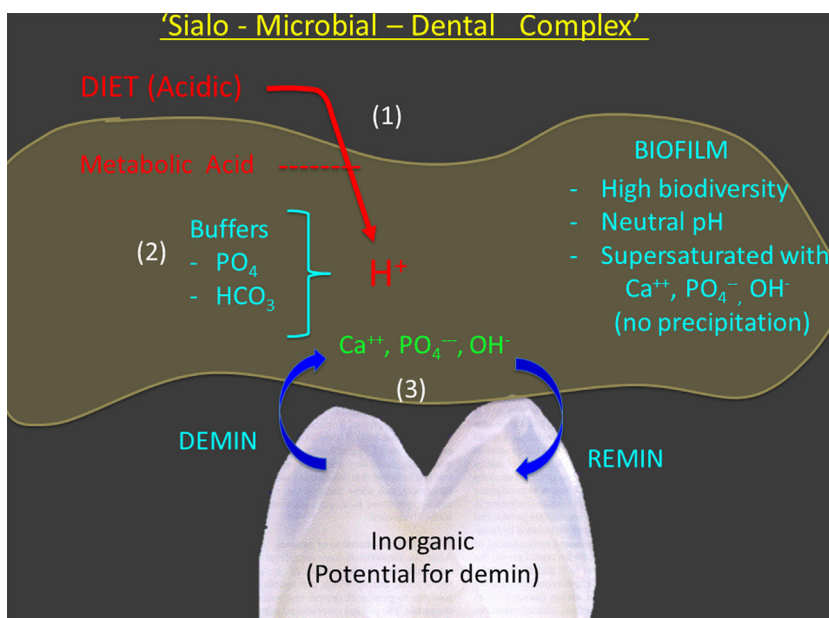
All the processes summarised above have evolved during a hunter-gatherer lifestyle and collectively are examples summarising an evolutionary strategy to protect the teeth from dissolution by dietary acids and, to a much lesser degree, metabolic acids. However, any physiological system has the potential to become pathological under certain conditions – where the evolved system becomes unbalanced, even within a hunter-gatherer setting. For example, systemic diseases such as rheumatoid arthritis and other auto-immune diseases can affect the quality and quantity of saliva and thereby compromise the buffering and remineralising capability of the system. This could be one of the reasons for the occasional reports of caries in some pre-contemporary hunter-gatherer populations.

#### 4. Imbalance of the oral environment

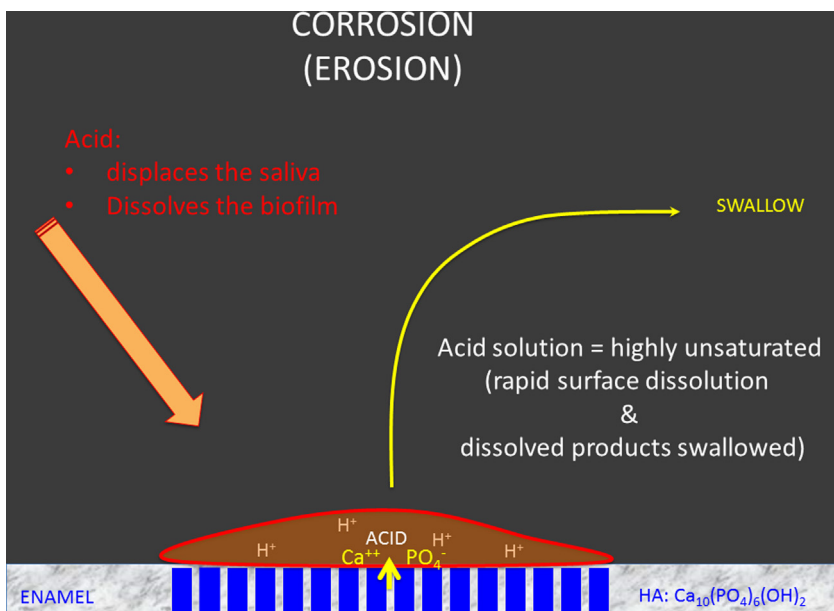
The sialo-microbial-dental complex can be regarded as a balanced system where the mineral maintenance of the teeth is attained (Fig. 1). However, there are a number of factors that will ‘unbalance’ the system resulting in disease. If the quality and quantity of saliva is compromised, the remineralising capability of the system will be compromised. In addition, if the makeup of the biofilm changes so that its resting pH drops, the balance tips towards demineralisation. Furthermore, a combination of a low resting pH and compromised saliva will further amplify and accelerate the demineralisation.

##### 4.1. Dental caries

The progressive increase in consumption of carbohydrates over the past 10,000 years has been responsible for much of the increase



**Fig. 1.** Stages of the demin-remin cycle: (1) dietary acid and, to a lesser degree, some metabolic acids cause a drop in resting pH. (2) Resting saliva (PO<sub>4</sub><sup>3-</sup>), followed by stimulated saliva (HCO<sub>3</sub><sup>-</sup>) buffer the acid. (3) If unsaturated conditions occur, demineralisation follows and, when the pH rises and saturated conditions are met, remineralisation repairs the damage.



**Fig. 2.** Excess consumption of dietary acids will dissolve the biofilm and displace saliva. Highly unsaturated conditions will cause rapid demineralisation without an opportunity for remineralisation to occur.

in population numbers across the globe. However this dietary change, together with the development of fermentation techniques for food preservation and the consumption of refined simple sugars since the industrial revolution, had an impact on the nature of biofilms worldwide (Adler et al., 2013). In addition, this dietary change from the hunter–gatherer lifestyle had a major impact on systemic, non-communicable diseases (e.g., diabetes, artherosclerosis, etc.) and, in particular, oral diseases (Hujöel, 2009; Ströhle et al., 2010).

The increase in frequency of consumption of complex carbohydrates and particularly simple sugars, challenges the oral microbiome. Firstly, bacteria (e.g., non-mutans streptococci and actinomyces) that produce very mild acidification of the biofilm gradually adapt via their own metabolic pathways to increase their production of metabolic acids. Similarly, mutans streptococci that are highly acidogenic and aciduric that exist initially in very low numbers, proliferate thereby further acidifying the biofilm (Takahashi and Nyvad, 2011). In addition, as a consequence, ‘good’ bacteria that produce alkalis and prefer living in alkaline conditions reduce in numbers and are eliminated.

Therefore, the introduction of and the increased frequency of consumption of refined simple sugars causes a drop in the resting pH of the biofilm, selecting for bacteria that produce acid and prefer living in acidic conditions. With the elimination of the ‘good’ bacteria there is also a significant drop in the biodiversity of the biofilm. Furthermore, in modern populations, there is excessive consumption of acid drinks instead of water that also adds to the acidification of the biofilm, thereby accentuating the development of a cariogenic environment. In summary, a change in diet has caused an ecological shift within the biofilm encouraging the development of an environment tipped towards net mineral loss and therefore dental caries. The pathogenesis of the carious lesion is a progression of continuous demineralisation of a tooth surface up to a point where the increasing porosity allows infiltration of bacteria (and more biofilm), further breakdown and a cavitated lesion.

#### 4.2. Dental erosion (corrosion)

Dental erosion or corrosion, is defined as the dissolution of dental tissues from acids of non-bacterial origin (Amaechi and Higham,

2005). This is a ‘modern day’ condition that results from the mastication of acidic foods and, in particular, the excessive consumption of acidic drinks such as soft drinks, alcopops, fruit juices, etc. This can also be considered a ‘lifestyle’ disease relating to contemporary modern culture and different to the Paelolithic past.

Biofilms are generally a physical barrier to acid, however, if the oral environment is overwhelmed with a very high frequency of acid consumption the balance within the oral environment tips towards net mineral loss. Dietary acids have the ability to displace saliva and when in excess are able to dissolve the biofilm and make direct contact directly with the tooth. Such highly unsaturated solutions cause rapid surface demineralisation that is permanent, where the dissolved raw products are swallowed and cannot be re-used (Fig. 2). After an acid challenge, the remaining yet damaged ends of enamel rods can be remineralised when saliva



**Fig. 3.** Glazed tooth surfaces showing active erosion on enamel.



**Fig. 4.** Labial erosion on a permanent central incisor.

re-contacts the tooth surface, however lost tooth substance will not be replaced.

Active dental erosion gives a tooth a glazed appearance (Fig. 3) and when dentine is exposed the tooth can become hypersensitive due to open dentinal tubules (Fig. 4). This is in contrast to exposed dentine caused by food abrasion where the dentinal tubules are covered by a mechanical smear layer over the surface. In addition, the eroded tooth surfaces are soft and are easily damaged with superimposed mechanical wear from mastication or tooth grinding.

Evidence of erosive patterns, described in the dental literature in contemporary populations, has not been reported in Palaeolithic hunter-gatherers. Although erosion must have occurred at a sub-clinical level in certain situations, generally its prevalence was so low that it could be considered to be insignificant. For example, systemic conditions resulting in chronic vomiting could have caused erosion in some individuals.

Finally, erosion in contemporary populations is also implicated with non-carious cervical lesions that present clinically with differing morphologies (Michael et al., 2009), one type being a wedge-shaped lesion. Although there are various opinions about the aetiology of the wedge-shaped lesion, such as excessive acid, mechanical wear (horizontal toothbrushing), and the mechanical flexure of teeth under load (abfraction), there is no doubt that these lesions are modern-day pathologies with a high prevalence in contemporary populations only.

## 5. Conclusion

The interactions occurring within biofilms is generally well understood and the current paradigm explaining the differences

between non-cariogenic and cariogenic biofilms ('ecological plaque hypothesis') has resulted from advances in cariology. However, these explanations have focussed on explaining the disease process without considering the evolutionary aspects of the oral system as a whole.

A shift in perspective is needed that acknowledges that oral biofilms have evolved symbiotically with the human host and together with other oral adaptations (e.g., salivary glands) to provide for the 'mineral maintenance' of teeth by physical and chemical means.

Oral diseases result when the evolved protective mechanism is overwhelmed by dietary and metabolic acids leading to net mineral loss. This imbalance has resulted from dietary changes over the past 10,000 years, culminating in the oral diseases that occur in epidemic proportions in today's populations.

Although further research is required to refine this paradigm, our new approach identifies directions for future dental clinical research. The 'utilisation' of the biofilm and the use of probiotics will have an impact in the preventive management of patients. Furthermore, from the perspective of evolutionary biology, one needs to question how dental structures of ruminants and other herbivores can survive in an acidic environment. We propose that the mechanisms are similar to Homo. In particular, the evolutionary function of cementum as a protective mechanism from erosion should be studied, considering that cementum covers the whole enamel crown in many species. Furthermore, the effects of 'unbalanced' biofilms on oral soft tissues also need to be considered from immunological and epigenetic perspectives.

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