Oral Biofilm, Saliva and BioXtra

An Overview

bioXtra

Introduction

The mouth is one of the major routes for the introduction of disease into the human body.
Increasing evidence shows that poor oral health can also be detrimental your general health and wellbeing. [1]

Maintaining healthy teeth and gums, therefore, is important not only for the sake of your appearance but also helps protect your whole body from disease.

Saliva's role in health and disease

The Role of Saliva

Saliva is the body's natural defence mechanism against the introduction of infection into the body via the mouth. [8]

Saliva protects the oral tissues in many different ways. A constant flow of saliva effectively eliminates microorganisms from the oral cavity whilst also containing many innate or acquired defence factors. These defence factors, through a variety of mechanisms, are capable of inhibiting microbial invasion, growth and/or metabolism.

The presence of saliva is essential to our health and wellbeing. However, the importance of this fluid is often overlooked or neglected by dentists and doctors

Saliva is produced mainly by the three paired exocrine glands
- The Parotid
- The Submandibular
- The Sublingual

The Location of the Salivary Glands

The glands are located in and around the mouth and throat. The major salivary glands are called the parotid, submandibular and sublingual glands.

Diagram 1
The glands all secrete saliva into the mouth: the parotid through ducts near the upper teeth, submandibular into the front portion under the tongue, and the sublingual through multiple ducts in the floor of the mouth.
In addition to these glands, there are a number of tiny glands called minor salivary glands located in the lips, inner cheek area (buccal mucosa) and extensively in other linings of the mouth and throat.
The salivary glands consist of many lobes that are separated by connective tissue called septa. Each lobe comprises clusters of gland cells that are organized into secretory structures called acini. The acinar cells secrete saliva, which is collected in small ducts that leave the lobules and open into the mouth.

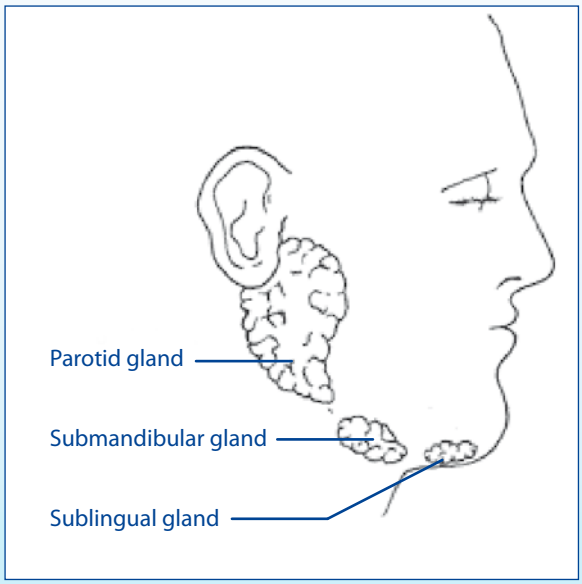
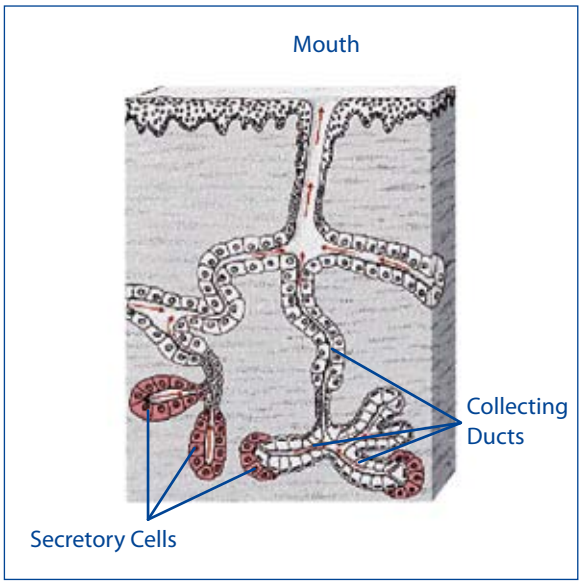


Diagram 2

The mouth has evolved for speaking, chewing, forming food boluses and swallowing, therefore the components of saliva are vital to facilitate all these oral functions, to maintain a neutral pH and to provide the correct ionic environment to protect teeth.



The Flow Rate of Saliva

Saliva is excreted in response to neurotransmitter stimuli. During most of the day the neurotransmitter release is low and a basal, or unstimulated, flow occurs. During food consumption there is a marked release of neurotransmitters, so stimulating greater secretion. Basal flow is considered to be a protective secretion whilst the greater stimulated flow is needed to facilitate ingestive processes and communication.

The resting flow rate for whole saliva averages about 0.3 to 0.4 ml/min. There is an enormous variation in flow rates for both basal and stimulated secretions.

The range of basal flow rates may vary between 0.08 and 1.83 ml/min whereas stimulated flow rates vary between 0.2 and 5.7 ml/min. Because of this heterogeneity it is difficult to assess the status of a patient's salivary gland function from a single measurement of flow rate. In the absence of complaints or visual evidence therefore, it is not easy to determine whether or not a patient has a salivary gland disorder.

However, if saliva production were assessed as a routine check, physicians or dentists would be able to establish a patient's normal flow rate and thus recognise where a decline was in progress. Such a scenario would allow early prevention treatments in order to limit the damaging consequences of salivary gland dysfunction.

The pH of Saliva

In health, the pH of blood is 7.4 and the pH of saliva is 7.4. Thus the pH of saliva parallels the extra cellular fluid.

The Significance of Salivary pH

Salivary flow dramatically decreases during sleep, creating a temporary xerostomia (dry mouth), which allows the accumulation of Gram-negative bacteria at the back of the tongue. During waking hours saliva is part of the normal defence mechanism of the oral cavity that washes away bacteria. [14] However, stagnation of saliva during sleep allows the pH to become acidic, which favors bacterial accumulation and enzymatic breakdown, making the saliva odiferous. [2, 15] On the other hand, mastication increases salivary flow, allowing a cleansing action of the oral cavity. Thus, increased salivary flow after wakening will allow morning breath to diminish.

The Importance of Acid and Alkaline Balance for Health?

Virtually all degenerative diseases including cancer, heart disease, arthritis, osteoporosis, kidney and gallstones, and **tooth decay** are associated with excess acidity in the body. While the body has a homeostatic mechanism that maintains a constant pH 7.4 in the blood, this mechanism works by depositing and withdrawing acid and alkaline minerals from other locations including the bones, soft tissues, body fluids and saliva. Therefore, the pH of these tissues and saliva can fluctuate greatly.
Cancer cannot exist in an alkaline environment. All forms of arthritis are associated with excess acidity. Acid in the body dissolves bones, and in the mouth dissolves teeth.

The Composition of Saliva

Saliva comprises around 99% water. The remaining 1% is mainly made up of large organic molecules such as *glycoproteins* and *lipids*, of small organic molecules such as *glucose and urea*, of electrolytes – chiefly *sodium calcium chloride* and *phosphates*.

Protective, Food and Speech Related Functions of Saliva

Lubrication	*Mucins, proline-rich glycoproteins, water*
Antimicrobial	*Salivary proteins:* lysozyme, lactoferrin, *lactoperoxidase, mucins, cystatins, histatins, secretory IgA, proline rich glycoproteins*
Mucosal integrity	*Mucins, electrolytes, water*
Cleansing	*Water*
Buffering	*Bicarbonate, phosphate ions*
Remineralisation	*Calcium, phosphate, statherin, anionic*
Digestion	*Amylases, lipase, ribonuclease, proteases*
Taste	*Water, gustin*
Speech	*Water, mucins*

The Functions of saliva

Saliva has many roles.

- **Lubrication and binding:** the mucus in saliva is extremely effective in binding masticated food into a slippery bolus that slides easily through the oesophagus without inflicting damage to the mucosa. Saliva also coats the oral cavity and oesophagus, and food basically never directly touches the epithelial cells of those tissues.

- **Softens and moistens dry food:** in order to be tasted, the molecules in food must be solubilised.

- **Oral hygiene & health:** The oral cavity is almost constantly flushed with saliva, which floats away food debris and keeps the mouth relatively clean. Flow of saliva diminishes considerably during sleep, allowing populations of bacteria to build up in the mouth - the result can be bad breath in the morning.

- **Antimicrobial factors,** Saliva acts, in a number of ways, as an essential protective mechanism protecting oral tissues and helping control orally transmitted pathogens. [8, 9] Salivary Antimicrobial factors can cut the cell walls of (explain) many bacteria to prevent overgrowth of oral microbial populations.

- **Wound healing factors** which help in gingival and mucosal tissues .

- **Initiates starch digestion:** in most species, the serous acinar cells secrete an alpha-amylase' which can begin to digest dietary starch into maltose.

- Provides **buffering** and fluid.

Recap

Saliva protects teeth and gums by:
- Neutralizing acid levels by maintaining a neutral pH (thereby reducing dental plaque formation)
- Controlling the balance of bacteria via its antimicrobial systems
- Flushing food and bacteria from the oral cavity (cleansing)
- Delivering calcium, phosphate and fluoride to the tooth surface to strengthen enamel (remineralisation)
- Providing high levels of oxygen in order to keep oral tissues healthy & fresh.

Saliva's Defence Mechanisms

Antimicrobial Systems
The oral cavity is a major route for the entry of exogenous often noxious materials, saliva also forms a first defence against their ability to cause disease in the body. Protection of oral tissues against the harmful effects of microorganisms by regulating the formation of oral flora is therefore one of saliva's most important functions.

This regulation is due to the activity of the salivary antimicrobial mechanisms, such as non-immune factors such as lysozyme, lactoferrin and lactoperoxidase in conjunction with other immune system components such as immunoglobulins[10], and growth (wound healing) factors which help maintain a healthy balance in the mouth, regulating the pH and thus encouraging healthy oral flora.

Of all the oral mechanisms the SALIVARY PEROXIDASE SYSTEM contributes the greatest to oral health. This natural protective mechanism plays a major role in maintaining a healthy eco-bacterial environment in the mouth.

The Salivary Peroxidase System

The Salivary Peroxidase System is responsible for producing one of the anti-bacterial agents present in healthy mouths.

The Salivary Peroxidase System comprises:
- Thiocyanate (a substrate present in saliva)
- Peroxidase (an enzyme)
- Hydrogen Peroxide (Produced by bacteria known as peroxigenic bacteria)
- Oxygen

The acid production of human dental plaque is also inhibited by the Peroxidase System.

Peroxidase
Salivary Peroxidase, often called 'Lactoperoxidase' because of its biochemical and immunological similarities to that extracted from bovine milk, is an enzyme of 77,000 MW.

Thiocyanate
The ion SCN^- is present in tissues and secretions. It is also present in every salivary gland.

Hypothiocyanite
This ion is a normal component of both human whole saliva and of parotid saliva. It binds to the sulfhydryl groups (SH) inhibiting the growth of bacteria. It can penetrate bacterial cells, inhibiting the uptake of glucose – the bacterial growth medium.

The salivary peroxidase system

Thiocyanate (SCN^-) + Hydrogen peroxide (H_2O_2) + oxygen (O_2)

Salivary Enzymes

The enzyme catalyses the oxidation of thiocyanate by the hydrogen peroxide in order to produce the ion

Hypothiocyanite ($OSCN^-$)

[Saliva's natural antibacterial agent]
(This molecule is a powerful antimicrobial against bacteria, viruses, and yeasts)

Diagram 3
The model above assumes that between periods of food ingestion, oral micro-organisms are relatively inactive. When food, such as carbohydrates, is ingested the bacteria become metabolically active and H_2O_2 in the oral cavity.

Reaction takes place between H_2O_2 in the presence of Thiocyanate, oxygen and salivary peroxidase enzymes giving an end-product known as Hypothiocyanite ions – $OSCN^-$ this is an inhibitory product. At lower concentrations, the more sensitive bacteria are inhibited. Finally, as H_2O_2 excretion continues and the inhibitory products accumulate, the peroxidogenic bacteria themselves are inhibited. Consequently, due to the reduction in H_2O_2 availability, the concentration of the Hypothiocyanite falls below the critical level. At this point, in the absence of adequate levels of this antibacterial agent, the inhibited bacteria may spontaneously recover from inhibition and the cycle resumes.

Effect of the Peroxidase System on Oral Bacteria

The oral cavity contains more than 350 species of microbes. These microbes are part of normal humans and are important to the health of humans.

The oxidation products of the salivary peroxidase system inhibit numerous species of oral bacteria.
Among these are the clinically important species such as Lactobacilli, Streptococci & Actinomyces.

Lactobacilli
Lactobacilli are highly acidogenic organisms, and early work implicated lactobacilli in the initiation of dental caries. Subsequent research has shown that they are associated more with carious dentine and the advancing front of caries lesions rather than with the initiation of the disease. Usually lactobacilli comprise less than 1% of the total cultivable micro flora. However, their proportions and prevalence may increase at advanced caries lesions both of the enamel and of the root surface.

Streptococci
Streptococci are most predominant microbes in mouth. These are sphere-shaped bacteria. They convert sugars & other carbohydrates into lactic acid. This acid then dissolves tooth enamel, eventually forming a cavity. Streptococcus mutans is the major culprit in forming cavities.

Actinomyces (Actinomyces viscosus) - They contribute along with other organisms to the pathogenesis of dental caries & periodontal disease. Many laboratory studies have employed *Streptococcus Mutans* as a test organism because of its central role in the etiology of dental caries. In fact, the growth, glucose uptake and acid production of this bacterium are strongly inhibited by the peroxidase system. For *Streptococcus mutans*, the inhibition is usually reversible.
Nevertheless, bactericidal action has been reported against some enteric bacteria such as *Escherichia Coli* and *Salmonella typhimurium*. This type of killing is enhanced by the increased permeability of the cell envelope.
On the other hand, the action of the system on the virus would be attributed to the effectiveness of $OSCN^-$ with the sulphydryl group responsible for the fixation of the viral envelope.
In addition to its antimicrobial effects, the oral peroxidase system also performs other important functions. By consuming hydrogen peroxide, the ensuing peroxidase catalysed reactions prevent the formation of toxic levels of H_2O_2 being excreted by peroxidogenic bacteria and host cells.
The susceptibility to of many species of oral bacteria to inhibition by the peroxidase system is partly dependent on their own H_2O_2 production, and partly due to the presence of an enzyme within the microorganism itself.

In short, the Peroxidase System has, compared to the other antiseptic molecules, the advantage of selecting and safeguarding the beneficial oral flora, of killing or inhibiting the harmful flora and thus regulating oral conditions.

Lactoferrin

Lactoferrin is an iron-binding protein found in milk, saliva and tears, and in other human and animal mucous secretions. Throughout the body it plays an important role in the primary defence system.
In the mouth lactoferrin is one of the most important antimicrobial agents where it promotes the growth of commensal flora by inhibiting the activity of unwanted bacteria, helping to prevent infection. Lactoferrin is antibacterial, antiviral and antiparasitic and plays a key role in the modulation of the inflammatory-immune response
Lactoferrin is a glycoprotein with a molecular weight of about 77,000 D. Each lacfoterrin molecule can bind two atoms of iron (Fe+++) tightly, but reversibly. The two iron binding sites are similar but not identical.
The binding of iron depends on the concomitant binding of synergistic anions, such as bicarbonate, which have an essential role in holding the metal firmly at its binding site on the protein.
Lactoferrin can exist either in an iron-free or iron-saturated state. It is evident therefore that the activity of Lactoferrin is closely linked to the presence of iron.

Origin of Lactoferrin in the Oral Cavity

Lactoferrin in pure salivary secretions mainly originates from serous glandular tissue, while mucous cells are devoid of lactoferrin. Immunohistochemical studies of parotid glands have shown abundant lactoferrin staining in serous acinar cells, and ductal segments stain irregularly for lactoferrin.
The serous cells of the sublingual glands show lactoferrin staining, whilst the mucous acinar cells of the submandibular and sublingual glands do not stain for lactoferrin. The serous acinar cells, unlike the mucous cells of the minor salivary glands, stain intensely for lactoferrin.

Polymorphonuclear leucocytes also contain lactoferrin, especially in their secondary or specific granules. Granular-derived lactoferrin may be released into saliva during the phagocytosis or disruption of these cells, causing high concentrations of lactoferrin in gingival crevicular fluid. Thus the lactoferrin of whole saliva originates either from the salivary glands or from leukocytes via gingival crevicular fluid.

Biological Functions of Lactoferrin

Action on Micro-organisms
Most bacteria need iron to metabolise. Lactoferrin's high affinity to bind to iron prevents this mechanism. Many studies have demonstrated a bacteriostatic effect. In some cases, *in vitro* tests have demonstrated a bactericidal effect on a wide range of micro-organisms including Gram positive and Gram negative bacteria aerobes and anaerobes, yeasts and virus (HSV-1), and human immunodeficient virus (HIV).

It has been proved that lactoferrin can also react directly on coliforms. The addition of lactoferrin in excess in relation with the quantity of iron present in the medium increased the inhibitory effect on the growth of *E. coli*. On the other hand, it was shown that the action of lactoferrin on a large number of micro-organisms such as *Yersinia, Campylobacter, Salmonella, Shigella, Staphylococcus aureus, Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans* is made by a specific interaction with each bacteria individually. In fact it was possible to purify the cell receptor of the *Staphylococcus aureus* able to bind to lactoferrin.

Lysozyme

Lysozyme was first discovered and described by Alexander Fleming in 1922. He also named a bacterium – Micrococcus lysodeikticus – most susceptible to this enzyme.

Lysozyme is an antimicrobial enzyme, which is present in almost all body tissues and secretions. In the mouth it is generally accepted to be of major significance in the prevention of dental caries and in controlling Candida.

Lysozyme can be found in high concentrations at those sites in the oral cavity, which harbour most bacteria. It acts in close synergy with Lactoferrin to control potentially harmful bacteria.

Lysozyme is present in many human tissues and practically all body secretions

Action of Lysozyme on Micro-organisms

Recent studies have revealed a wide range of additional mechanisms of lysozyme against oral bacteria. Lysozyme has also been found to be capable of:
• Aggregating bacteria
• Inhibiting adherence to saliva-coated hydroxyapatite
• Inhibiting the co-aggregation between oral bacteria

Bacterial aggregation is probably of minor importance in the oral cavity. However, Lysozyme, in contrast to other saliva agglutins, can aggregate these bacteria at low temperature. For example, Mutans streptococci exposed to physiological levels of Lysozyme show de-chaining of streptococcal chains and areas of cell wall dissolution without cell lysis.

Several findings suggest that at least part of Lysozyme's activity against bacteria and Candida is due to its cationic nature. This antimicrobial activity has been attributed to an increase in membrane permeability followed by electrolytic and osmotic changes within the cell. These membrane changes induce a number of physiological and metabolic changes within bacterium.

Normally K+ ions accumulate in bacterial cells against a concentration gradient when glucose is available. Treatment of bacterial cells by Lysozyme may lead therefore to a depletion of intracellular K+, which further results in a marked drop of membrane potential, a loss of transport mechanisms over the membrane, inactivation of K+ dependent enzymes, loss of turgor pressure and finally, cell death. Lysozyme purified from human saliva also decreases glucose incorporation and acid production in S. mutans cells *in vitro* and *in vivo*.

Immunoglobulins (Antibodies)

In combination, these factors can help control disease-causing organisms in the mouth. They can also limit the formation and adhesion of bacteria to the mucous membranes in the mouth, so helping avoid cell damage and assisting the mouth's natural healing processes.

Antibodies

The immune system consists of immunoglobulins, which are produced by activated B-lymphocytes. [1] Immunoglobulins (Ig) are divided into five classes: IgG, IgA, IgM and IgD. Serum contains mostly IgG, some IgA and IgM but only minor amounts of IgE and IgD.

The proportions of different Ig classes in saliva rank in order IgA > IgG > IgM > IgD > IgE.

Immunoglobulin A (IgA) is the predominant immunoglobulin in all human external secretions, such as colostrum, milk, tracheobronchial secretions, saliva and genito-urinary secretions. [3] Ninety-five percent of the salivary IgA is produced by the local plasma cells in salivary glands and only a minor part is leaked from serum. [4] When calculated by total daily output, secretory IgA (sIgA) is principally produced and secreted from the parotid glands. [5] The submandibular glands, however, contain about twice as many IgA-producing cells as the parotid glands, [6] and the minor salivary glands have even higher concentrations of IgA-producing plasma cells. [7]

No IgA can be detected in saliva at birth, but adult level of IgA develops in early childhood. [8] The oral levels of IgA are dependent on salivary flow rate, and therefore decline in salivary secretion also results in decreased sIgA levels. [9] The concentration of IgA in human saliva does not decline with aging. [10]

The protective role of sIgA is based on several mechanisms, such as neutralization of toxins and enzymes [11] and inhibition of adherence of oral microorganisms of tooth surfaces. The antimicrobial capacity of sIgA is even elevated by synergistic action with many innate factors, i.e. lysozyme, lactoferrin and lactoperoxidase. [12]

In subjects with immunodeficiencies, glandular development of IgM immunocytes may take place as a compensaory mechanism [23] and IgM concentrations are usually elevated in salivary secretions of such individuals. [24]

Saliva's Antimicrobial and Wound Healing Action

Wound healing factors are naturally occurring substances in saliva. They help in wound healing of gingival and periodontal tissues damaged by bacteria in biofilms.

Diagram 4
A SINGLE BIOFILM LAYER
In isolated, planktonic form biofilm bacteria are aerobic and not pathogenic

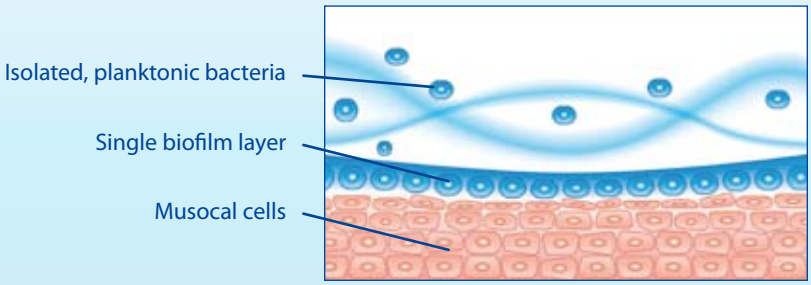


Diagram 5

AGGREGATION OF PATHOGENIC BACTERIA IN MULTIPLE BIOFILM LAYERS

As subsequent layers of biofilm form, bacteria in the inner layers become anaerobic, and pathogenic, causing inflammation on mucosal surfaces.

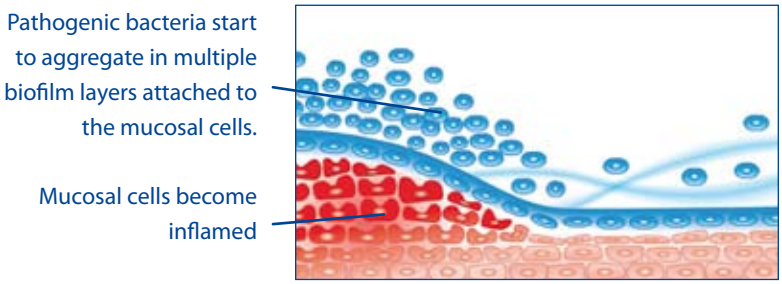
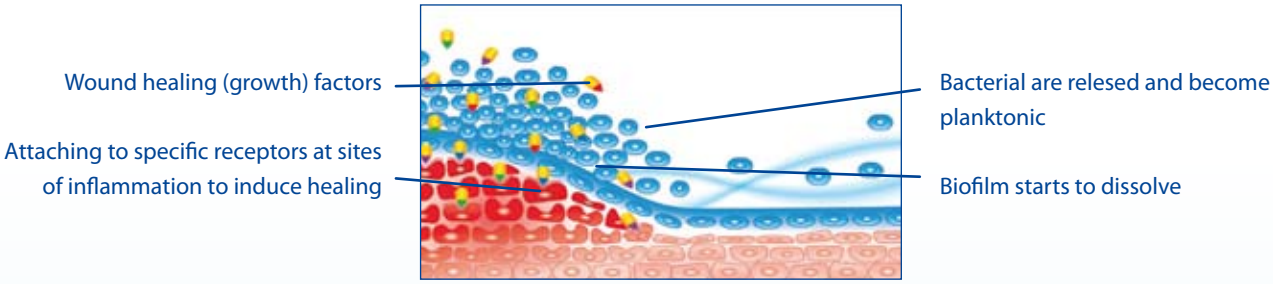


Diagram 6

NATURAL WOUND HEALING FACTORS

Inflamed oral cells produce, on their surface, specific receptors which relate to wound healing factors present in saliva. By attaching to their receptor the wound healing factors help repair damaged cells, modifying the oral environment and encouraging the removal of biofilm.



Oral bacteria in a healthy mouth are present either individually, known as isolated or planktonic bacteria (**Diag. 4**) or in organized in a single layer on teeth and gums (biofilm). Dental plaque is a form of biofilm.

Where there is an imbalance in the oral environment, bacteria, which are opportunistic, will start to accumulate on the mucosal surfaces. The inner layers of this biofilm comprise anaerobic bacteria which, when attached to gums and teeth, can cause dental plaque and oral inflammation. If left unchecked this often leads to progressive oral disease (**Diag. 5**).
At the site of infection or inflammation, wound-healing receptors appear. Wound healing (growth) factors present in natural saliva attach to their specific receptors to provoke the natural healing process. (**Diag. 6**)

Recap

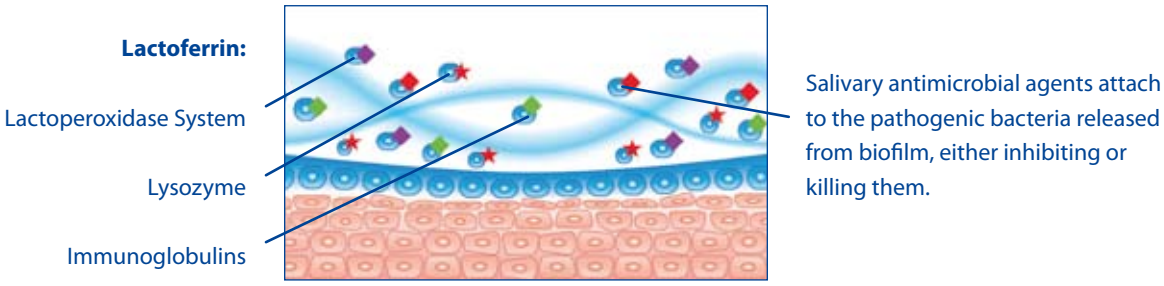
Wound healing factors are naturally occurring substances in saliva. They help in wound healing of gingival and periodontal tissues damaged by bacteria in biofilms. Inflamed oral cells produce specific receptors. Wound healing factors penetrate through biofilms. Wound healing factors have a strong affinity for these specific receptors found at the site of inflammation. These factors attach to their specific receptors. They repair damaged cells and encourages removal of biofilms.
There are 5 types of wound healing factors found in saliva:

- Epidermal growth factor
- Transforming growth factor
- Platelet derived growth factor
- Fibroblast growth factor
- Insulin-like growth factor I & II

Diagram 7

SALIVA'S INHIBITORY ANTIMICROBIAL SYSTEMS

Natural mechanisms maintain the mouth's natural healing processes, discouraging the attachment of biofilm bacteria on oral surfaces. The detached biofilm bacteria become isolated (planktonic)



It has been demonstrated that the salivary molecules lactoferrin, lysozyme and immunoglobulins can react on the microorganisms forming the outer layer of the biofilm and on isolated bacteria. (**Diag. 7**) Consequently they are not present to react against the bacteria located inside the biofilm. Similary, the active agent of the Salivary Peroxidase System, hypothiocyanite ions ($OSCN^-$), acts on the bacteria located on the external surface of the biofilm and against isolated bacteria. Their lack of efficacy against the bacteria located inside the biofilm is due to the dilution factor the molecules have to support when penetrating the biofilm.

Hence saliva's wound healing factors (**Diag. 6 & 7**) along with its antibacterial factors help in removing biofilms and in the repair of gingival and periodontal tissues, keeping the microbial flora of the mouth in check and thus maintaining a healthy oral ecosystem.

Biofilm Bacteria

In nutrient-limited ecosystems, such as the aquatic environment, bacteria have a marked tendency to attach to surfaces and initiate the formation of biofilm. These biofilms are also a severe problem in medical science, such as in oral health where they can cause dental plaque and oral disease.

Bacteria in the mouth are present either individually (isolated or planktonic bacteria), or are found in organised layered masses (biofilm bacteria). More than 70% of bacteria in the mouth are found in biofilm masses. [2]

Biofilm bacteria behave in a more complex way than isolated bacteria due to their ability to exist in bacterial communities. [3]

These biofilm communities are composed of a network structure of micro-colonies from a number of species. In medical and dental science biofilms pose a severe problem to health. This is due to the ability of the organised bacterial colonies to attach to a large variety of cellular or artificial surfaces (such as implants, catheters etc). [4] In the mouth biofilms attach to soft and hard tissue causing dental plaque, and may lead to progressive periodontal disease. [5]

Formation of Biofilms

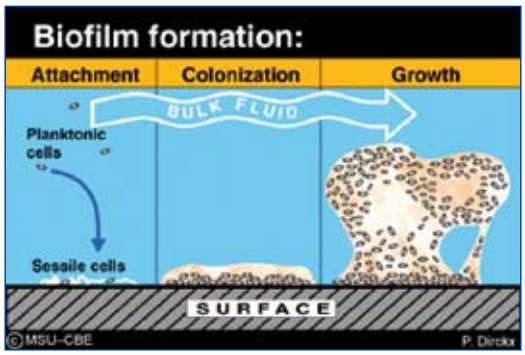


Diagram 8

1. Isolated or Planktonic bacteria attach to mucosal surface
2. Form colonies under certain conditions
3. Grow further to form network of bacteria, fungi, protozoa or viruses.

Two types of Biofilms

In oral biofilm, only the uppermost layer is composed of non-aggressive, aerobic bacteria. As new layers form on the surface, the aerobic bacteria, starved of oxygen, adapt to this new condition and become anaerobic. In this form, such bacteria can start to cause damage to the mucosal surfaces to which they adhere. [6] Such damage can be more prevalent in patients suffering dry mouth
Single-layered Biofilm: This layer, which is essential to protect teeth and gums, forms on the enamel or on oral mucosa, A single layer biofilm contains aerobic microbes which are non-pathogenic. A single layer of biofilm does not pose a problem.
Multi-layered Biofilms: under conditions which favour their proliferation, bacteria can accumulates in multiple layers forming multi-layered biofilms. Where there is an accumulation of anaerobic, aggressive and pathogenic microbes dental plaque, gingivitis, periodontal disease/ inflammation will be more prevalent.

Although some salivary agents are able to bind to bacteria preventing their adhesion to mucosal cells, they are not able to remove the biofilm layers.[11] Due to the fact that the biofilm matrix is a collection of microcolonies the ability of the antimicrobial agents contained in saliva to react significantly on the bacteria organised in biofilms depends largely on the thickness of the biofilm.

In vitro studies using *Staphylococcus epidermis* and *Staphylococcus aureus* showed isolated (planktonic) cells to be significantly more sensitive to the Lactoperoxidase System than were the biofilm cells. [12]

The test results on the total bacteria count confirmed that anaerobic biofilm cells are more resistant than isolated cells. This is believed to be due to:

- The physical protection of the biofilm matrix preventing large molecules such as Lysozyme, Lactoferrin and Immunoglobulins from penetrating the layers. [13]
- An altered physiology of the bacteria in biofilm. [14]
- The dilution of the antibacterial agents as they try to filter the biofilm layers. [15]

It has been demonstrated that large molecules such as lactoferrin, immunoglobulins and lysozyme are not able to penetrate biofilm due to their size. [13] Several studies, performed on saliva samples, showed that the antimicrobial activities of the Salivary Peroxidase System, Lactoferrin, Lysozyme and Immunoglobulins did not significantly affect the accumulation or acidogenicity of biofilm bacteria. Similar results were obtained when studying the effect of both antibiotics and antiseptics. [7, 13, 15, 16, 17]

This is mainly due to the presence of bacteria, organised in biofilms, which do not react to the antimicrobial agents in the same way as when they are detached and isolated.

The underlying layers of anaerobic biofilm bacterial cells may, therefore, be significantly less affected by the inhibitory effect of the Salivary Peroxidase System, even though under aerobic conditions such cells would have limited resistance. [16]
Furthermore, as Thiocyanate and Hydrogen Peroxide (part of the Salivary Peroxidase System [37] diffuse through the biofilm layers their antimicrobial action decreases compared to their effect on isolated cells - suggesting that the underlying cells of the biofilm escape the antibacterial activity of the Salivary Peroxidase System, unless the biofilm can be released from the mucosal surface..
Such evidence may explain the fundamental difference in susceptibility between bacteria in established biofilm matrix and isolated bacterial cells. [16]
This evidence suggests that salivary antimicrobial enzymes alone may be ineffective against such micro-organisms - mainly due to the layered structure of the biofilm and their inaccessibility.
The inner, anaerobic cells of the biofilm, therefore, will escape the antibacterial activity of the lactoperoxidase system, unless the biofilm is released from the mucosal surface and rendered planktonic.
Such evidence may explain the difference in susceptibility between the bacteria in biofilm and the planktonic cells.

Wound Healing (growth) factors

Nevertheless, there are other important natural mechanisms which encourage biofilm bacteria to detach from mucosal surfaces. In fact, whilst the inner bacterial layer is attached to the mucosal cells they secrete exolipopolysaccharides (EPS), commonly known as 'virulence factors. [20, 21] causing inflammation. The affected cells counter-react by producing 'growth factors'. Where inflammation is present, these growth factors are also present in saliva in large concentrations. [22-30] helping to repair damaged, inflamed cells and eliminate bacterial attachment. The detached bacteria then become isolated and susceptible to the other antimicrobial systems of the molecules. [22, 23, 30]
(See *Saliva's Antimicrobial and Wound Healing Systems* above)

A Lack of Saliva
Dry Mouth

The mouth is a major route for the introduction of disease into the body. Saliva's natural defence mechanisms have evolved to counteract the potential effects of harmful bacteria, and maintain the mouth's natural eco-system.
In a healthy mouth the continuous combined action of salivary defence systems, such as the Salivary Peroxidase System, Lactoferrin, Lysozyme, Immunoglobulins and Growth Factors, combined with good oral hygiene, helps maintain a neutral pH and creates an oral environment where harmful bacteria are inhibited, preventing the formation of biofilm.
The importance of saliva can be best demonstrated by studying those individuals suffering the effects of **Dry Mouth.**

Dry Mouth is known in medical terms as Xerostomia. It is a condition caused by a decrease in the amount of saliva produced. In some cases the flow of saliva may cease altogether.

Although Dry Mouth is not widely recognised, research suggests that there may be as many as 1 in 5 people suffering the effects of Dry Mouth at some time in their life.

One of the major factors contributing to poor oral health, therefore, is a reduced salivary flow which almost always leads to progressive oral diseases, difficulties in eating and speaking, and to a variety of symptoms including a feeling of 'burning mouth'. [8, 9, 18]

Apart from the associated discomfort caused by a persistent dry mouth, a prolonged reduction in saliva flow means the loss of natural protection and the potential development of infection. This poses a real threat to the general and oral well being of dry mouth patients. [1, 8]

The Effects of a Lack of Saliva

The feeling of having a Dry Mouth is very subjective and can occur quite normally from time to time. However, a person experiencing a feeling of dryness regularly or constantly will find it difficult to carry out normal day-to-day mouth functions such as eating, swallowing and speaking. Their sleep patterns will also be severely affected.
In addition, there will be a gradual deterioration in the condition of the teeth and gums, an inability to taste food properly, a persistent bad breath and in some cases, a feeling of burning in the mouth.

Denture wearers often find that their dentures become less comfortable and more difficult to keep in place.
At night, especially, patients may find themselves waking frequently with a constant need to lubricate the mouth.
The consequences of prolonged Dry Mouth, therefore, can include progressive periodontal disease, dental caries, tooth loss, mucosal trauma, persistent bad breath, and infections in the upper gastro-intestinal tract. [1, 18]
Whilst these symptoms can vary from one individual to another there is no doubt that a constantly dry mouth is inconvenient, distressing, painful and embarrassing to the point that enjoyment of life can be severely reduced.

The Causes of Dry Mouth

Any alteration in salivary gland function may create a decrease in saliva flow. This condition, known as 'Dry Mouth', is considerably more prevalent than commonly recognised. Apart from the discomfort caused by a persistent dry mouth, a prolonged reduction in saliva flow means the loss of natural protection and the potential development of oral infection due to the increase in colonised bacteria. Oral bacteria also pose a significant threat to general health and wellbeing

Dry Mouth can be a **temporary** or **long-term** condition.

There are a number of causes of temporary Dry Mouth, the main one being the taking of medications. In fact, there are as more than 400 commonly used medications, which may cause Dry Mouth as a side effect. (See Appendix 1)
Long-term Dry Mouth may be irreversible and can be due to medical treatments such as radiotherapy or head & neck surgery, as well as by a number of other disorders and autoimmune diseases. (See **Table 4** below)

Table 4

Long-term causes of Dry Mouth	Temporary causes of Dry Mouth
• Radiotherapy to the head & neck.	• Anxiety
• Head & neck surgery	• Stress/Depression
• Diabetes Mellitus	• Vitamin Deficiency
• Sjogrens Syndrome	• Medications
• Systemic Lupus Erythematosis [SLE]	• Mouth-breathing
• Rheumatoid Arthritis	• Hypertension
	• Hormonal changes
	• Smoking

Easing the Effects of Dry Mouth

Momentary relief can be achieved by sipping water. But because saliva is so important Physicians and Dentists often recommend sugar-free sweets or chewing gum. This is to try to stimulate the flow of natural saliva so that the mouth can benefit from its own natural protective mechanisms.

However, none of these will provide a feeling of sustained moisture, nor will they act in the same way as natural saliva does in maintaining oral health, comfort and freshness.
In the case of Dry Mouth, evidence suggests the continuous presentation of salivary antimicrobial enzymes and other essential salivary components can provide an important contribution towards improving the quality of life
In a dry mouth, where biofilm has become well established, the replacement of saliva's protective systems can be important in helping to avoid the consequences of a lack of saliva. [1, 8]
The concept of these biofilm bacterial growth patterns has, in the past, rarely been considered in developing products to control or limit bacterial activity. Research has been conducted using models with isolated bacteria, which are more fragile and easier to eliminate than biofilm bacteria. However, some studies have shown that the or local concentration of antibiotics necessary to eliminate biofilm bacteria would need to be 1000 times higher than for isolated bacteria. Such concentrations may be very difficult to achieve and may be harmful to the subject. [7]
Moreover, Lactoferrin and other antimicrobials such as Lysozyme and the Peroxidase sStem have, in the past, been added to oral health products to help counteract the effects of harmful bacteria. Nevertheless, these supplements have not resulted in significant changes in oral plaque or in relieving other periodontal problems. This is considered to be due to the inability of these molecules to penetrate biofilm masses in adequate concentration to have a significant effect on the host bacteria.

Effectiveness against biofilm bacteria requires an approach which closely follows the antimicrobial AND the wound healing action of natural saliva.

It is well known that milk is a rich source of health-promoting components. Resulting from specialist research and technology Bio-X Healthcare has developed innovative oral care products which harness natural molecules, present in untreated milk and in milk by-products. All BioXtra ingredients are extracted from cow's milk or from Colostrum . Colostrum, which is also called 'first milk', is one the oldest and most exceptional foods that can be found in nature. Colostrum is the milk of any mammal in the first 24 hours after birth. The effective substances that are present in this first milk are found nowhere else in nature in such high, perfectly balanced concentrations. In vivo, these molecules have been shown to be effective in facilitating the dispersal of biofilm bacteria and provoking the mouth's natural healing mechanisms.

Milk's protective molecules

The antimicrobial function of Bio-X Healthcare's active molecules is the same as that present in saliva, tears, breast milk and other human and animal secretory fluids.

LACTOFERRIN
- Key part of the primary defence system
- Iron binding, anti-oxidant
- Anti-bacterial, antiviral, antiparasitic
- Modulates inflammatory-immune response
- Promotes healthy bacteria

LYSOZYME
- Antimicrobial protein
- Inhibits the growth of pathogenic bacteria
- Works in synergy with Lactoferrin
- Found at bacterial sites in the mouth
- Regulates caries and candida

LACTOPEROXIDASE
- Reacts with substrates to produce saliva's natural antibacterial agent- hypothiocyanite
- Minimises harmful bacteria
- Prevents tooth loss, gum disease, bad breath

IMMUNOGLOBULINS
- Important proteins produced by the body's immune system
- Essential defence mechanism
- Increases the body's natural resistance to infection
- At the first signs of infection, specific antibodies target and attack the micro- organisms
- Present in saliva and crevicular fluid

COLOSTRUM EXTRACT
- Contains immune and non-immune factors, anti-bacterial molecules, vitamins and minerals
- Activates the body's natural protective mechanisms
- Helps protect health and vitality
- Helps control disease-causing organisms Can limit the formation and adhesion of anaerobic bacteria
- Helps avoid cell damage
- Provokes the mouth's natural healing processes.

The BioXtra Oral Care Programme

ULTIMATE ORAL CARE

Clinically Proven to Ease the Symptoms and Effects of Dry Mouth

In the past several products have been developed which offer a constant daily concentration of antimicrobial agents such as $OSCN^-$, Lactoferrin and Lysozyme in an attempt to promote a stable, balanced oral environment.

However, studies, performed on saliva samples, showed that the antimicrobial activities of these products - the Lactoperoxidase System, Lactoferrin and Lysozyme - did not significantly affect the accumulation or acidogenicity of dental plaque. Moreover, the number of plaque-derived mutans streptococci, total streptococci, lactobacilli and total anaerobes did not change significantly during the experimental period. This confirms earlier observations where ATP analysis showed that the peroxidase-system-generated HOSCN/ $OSCN^-$ is usually bacteriostatic rather than bactericidal.
This is mainly due to the presence of bacteria organised in biofilms, which do not react to the antimicrobial agents in the same way as when they are detached and isolated.

In oral biofilm, only the uppermost layer is composed of aerobic (planktonic) bacteria. As new layers form, these aerobic bacteria become anaerobic and can start to cause damage to the mucosal surfaces to which they adhere. As has been explained earlier, antimicrobial enzymes are usually ineffective against such micro-organisms due to the layered structure of the biofilm.
Nevertheless, small molecules able to interact with damaged epithelial cells can liberate the biofilms, thus detaching pathogenic micro-organisms from the epithelial cell. Such isolated cells become more accessible to antimicrobial proteins and enzymes.

New concept
The BioXtra products - a new generation and concept in oral care for dry mouths - have been shown to be effective in helping disperse biofilm, rendering it more susceptible to the activity of the anti-microbial agents present in the formulation.
BioXtra's unique formulation mimics not only the essential Salivary Peroxidase System, but also introduces other naturally occurring salivary components, to help supplement the mouth's natural anti-bacterial non-immune and immune mechanisms.

The BioXtra products, based on a tried and tested patented formulation, combine salivary protective systems with **Colostrum Extract** and have been proven to be effective in releasing the biofilm bacteria from the mucosal surfaces in order to make them more accessible to the antimicrobial effects of the Lactoperoxidase System, Lactoferrin and Lysozyme. Moreover, Immunoglobulins present in the **Colostrum Extract** also bind to the isolated bacteria inhibiting a re-adhesion to the epithelial cells so helping to regenerate and repair damaged mucosa.

The active ingredients in BioXtra, derived from milk and milk by-products, have been carefully selected to create a formula which works optimally to provide a regular supplementation of essential oral components. [31] Any ingredients known to lessen the effectiveness of the formulation have been excluded. These include, amongst others, Sodium Lauryl Sulphate, [33, 34] Sodium Fluoride, [35, 36] alcohol and menthol.

In the mouth, concentrations of salivary antmicrobials can vary as a result of factors such as diet, stress, vitamin deficiency, ageing, medications, systemic diseases and medical treatments.
In order to optimise the protective activity of natural saliva, therefore, salivary compounds have been added to the BioXtra range rendering them particularly appropriate in the case of dry mouth, where little or no natural saliva protection is present.

In addition to its unique antimicrobial action, BioXtra's patented **4-phase** action works optimally to provide an immediate feeling of moisture whilst promoting mouth comfort, oral health and freshness. Shown in-vivo to be effective in dispersing biofilm bacteria and provoking the mouth's natural healing mechanisms, BioXtra is recommended and prescribed by physicians for the prophylactic care of dry mouth. BioXtra is also beneficial to anyone who has difficulty in maintaining good oral hygiene.
Regular, daily application of the BioXtra products provides a unique oral care regime that eases the effects of a lack of saliva and helps promote oral health by helping maintain a natural balance in the dry mouth.

BioXtra's Active Ingredients

Lactoferrin, Lysozyme, Lactoperoxidase System, Colostrum Extract (immunoglobulins and growth(wound healing) factors), Moisturisers, Xylitol, Minerals, Aloe Vera, Essential Oils.

Mechanisms of BioXtra Active Ingredients

BioXtra's natural mechanisms maintain the mouth's natural healing processes, discouraging the attachment of biofilm bacteria on oral surfaces. The detached biofilm bacteria become isolated (planktonic) and subject to action of BioXtra's antimicrobial systems. See diagram below)

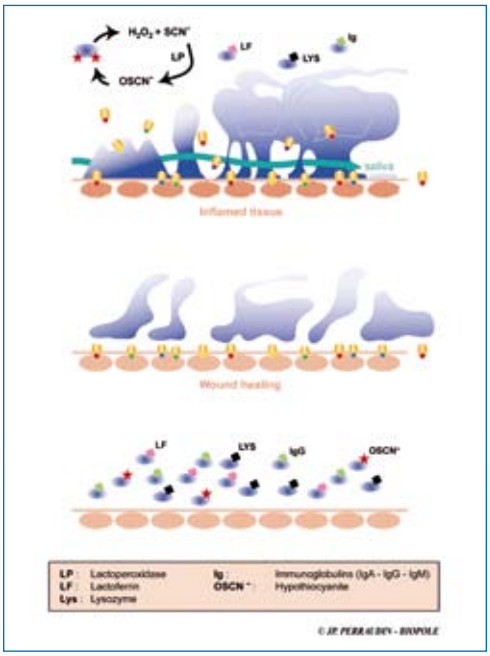


Diagram 9

The fundamental action of the BioXtra 'Programme' is to mimic saliva in its moisturizing AND its protective functions. There are many saliva substitutes which offer moisturisation. However BioXtra is the ONLY range of products to closely replicate many of the roles of saliva and to have an effect against biofilm bacteria.

In losing the quantity and quality of saliva, a sufferer will experience not only a feeling of dryness, but also, a gradual deterioration in oral health. This deterioration will lead very quickly to tooth decay, tooth loss, oral inflammation and other systemic disorders, as well as functional oral problems.
Consequently the individual products which comprise the BioXtra range have an important interaction, one with the other, in improving quality of life for these sufferers.
This holistic approach to the problems associated with Dry Mouth is part of our mission statement:

Moisture, Comfort and Protection for improved Quality of Life

The Bioxtra products are UNIQUE in their moisturising and antimicrobial action, working synergistically to improve mouth comfort and mouth health by controlling the bacteria which cause pH imbalance, leading to inflammation, gum disease, dental decay, tooth loss, plaque and biofilm, bad breath and reduced quality of life.
Whilst the gel and gelspray offer the sufferer an immediate feeling of moisture and comfort, it is the combined action of the oral hygiene products with the saliva substitute products which offer the best long term solution to the sufferer.

The BioXtra Range – Ultimate Oral Care

For Optimal Oral Hygiene

Mild Toothpaste – 50 ml
In the case of dry mouth, a regular, thorough oral hygiene is of essential.

A good brushing technique is important to ensure debris removal and to dislodge plaque. However, for the dry mouth sufferer this normal, daily task can be less effective than is the case in a normal mouth.
Moreover, the physical activity of tooth brushing amongst this group is often an unpleasant and painful process, discouraging the sufferer from being as thorough as is required. At the same time, a reduction in saliva flow means essential salivary protective factors will be less efficient.
Dry Mouth sufferers, therefore, can often find few solutions to maintaining good oral hygiene in the long term.

BioXtra Mild Toothpaste helps replace and supplement saliva's natural protective factors in addition to providing fluoride (sodium monofluorophosphate) and xylitol to help guard against caries. [32] At the same time, its gentle, mild-flavoured formulation makes it easily tolerated by sore mouths, encouraging regular, consistent use.

Formulated without foaming agents, which are counteractive to salivary activity and can contribute to mouth ulcers, [33, 34] BioXtra Mild Toothpaste is especially gentle, yet proven to be effective in helping maintain the mouth's natural balance, in the way nature intended

- Natural antimicrobial formulation
- Active within 5 seconds and during 5 hours
- Rapid, prolonged effect against plaque-causing, biofilm bacteria
- Cleanses without burning or stinging
- Especially gentle on dry or sensitive mouths
- Maintains the mouth's delicate eco-system
- Discourages mouth ulcers
- Promotes fresh breath

Alcohol-free Mouthrinse – 250 ml
Regular use of BioXtra Mouthrinse after brushing helps boost the action of BioXtra Toothpaste.

BioXtra Mouthrinse is helpful in keeping the mouth feeling moist, fresh and comfortable, whilst its alcohol-free formulation means it is extra gentle on sore mouths.

- Prolongs the action of BioXtra paste
- Supplements saliva's natural protective mechanisms
- Mild, non-burning formulation
- Reduces dental plaque formation and gum inflammation
- Refreshes and moistens the mouth
- Alcohol-free for added comfort and protection.
- Safe as a long-term prophylaxis

For Long-Lasting Saliva Substitution

Moisturising Gel – 50 ml

When there is a lack of saliva the sufferer craves a replenishment of moisture. However, water offers nothing more than a momentary alleviation of the feeling of dryness and is not sufficient to ease the symptoms or to prevent the gradual deterioration in oral health resulting from a prolonged lack of saliva.

BioXtra Moisturising Gel not only eases the sensation of dry mouth for hours at a time, especially at night, but at the same time it delivers a combination of important anti-bacterial and antimicrobial components which help normalise conditions in the mouth.

- Immediate, long-lasting relief of dry mouth, especially at night
- Forms a moisturizing barrier over dry oral surfaces
- Slow-release of moisture and active mechanisms.
- Improves mouth function.
- Provokes the mouth's natural healing processes
- Promotes a healthy, comfortable mouth
- Ideal under dentures

Gelspray – 50 ml

The lightly viscous formula coats dry mucosa to give an immediate sensation of lubrication and comfort. At the same time, the formulation helps encourage the flow of gingival crevicular fluid, helping prolong the sensation of natural moisture.

BioXtra Gelspray is a convenient alternative to BioXtra gel for daytime use, and is a practical solution in mouth care for immobile, or bedridden patients.

- Instantly relieves dry mouth
- Helps improve the mouth's moisture balance temporarily
- Non-drying formula coats mouth surfaces, lips and throat
- Feels like natural saliva.
- Natural salivary components help promote better oral health
- Convenient and easy to use during the day

How to use the BioXtra Oral Care Programme

1. For optimal oral hygiene: Brush teeth with BioXtra Mild Toothpaste after every meal. After brushing, rinse with BioXtra Alcohol-Free Mouthrinse or use at any time during the day for extra freshness.
2. For long lasting mouth moisture: Apply BioXtra Moisturising Gel liberally to the gums and tongue whenever necessary, especially at night, or during the day, spray the mouth with BioXtra Gelspray for lasting comfort.
3. To stimulate saliva: Chew BioXtra Sugar-Free Chewing Gum or suck a BioXtra Tablet as often as required.
For maximum effect use the BioXtra products together as a daily oral hygiene routine.

Indications

Indicated for use in patients suffering with Sicca Syndrome or Xerostomia resulting from Medications, Auto-immune disease, Radio- or Chemotherapy, Head & Neck surgery, Palliative Care.

Reasons to prescribe/recommend BioXtra

- Non-chemical management of oral infection
- Discourages biofilm formation
- Introduces protective salivary immune and non-immune factors
- Assists the mouth's natural healing processes
- Controls acid-causing bacteria, regulating pH
- Helps reduce candida
- Improves oral health
- Maintains a moist, fresh, comfortable mouth
- Helps prevent bad breath Easy to use and to tolerate
- Safe for long-term use
- Can be used by denture wearers and diabetics
- Improves quality of life and restores personal dignity ***Conclusions***

Used regularly, the **BioXtra** range of products offers a major advance in oral care helping lessen the long-term oral effects associated with dry mouth.

To the growing sector of the population experiencing dry mouth, **BioXtra** can help reduce the progressive symptoms associated with a long-term lack of saliva by supplementing the antimicrobial systems and immune factors normally present in saliva and promoting the maintenance of commensal flora in the mouth.

The products can also be beneficial to any individual experiencing difficulty in maintaining good oral hygiene.

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Appendix 2

Commonly Prescribed Drugs which can cause Dry Mouth

List of few important Xerogenic medications

Analgesics:	Pentazocine.
Anorexiant:	Fenfluramine
Antalgiques- Anti-inflammatory:	Ibuprofen
Anti-anxiety:	Diltiazem, Alprazolam
Anti-Asthma :	
Anti-depressant:	Amitriptyline, Imipramine
Anti-diarrheic:	Loperamine
Anti-histamine:	Chloophenamine, Diphenhydramine
Anti-epileptic:	Carbamazepine
Anti-hypertensive:	Atenolol-Nifedipine, Enalapril, Methyldopa, Prazosine
Anti-parkinson:	Levodopa
Anti-spasmodic:	Phenobarbital
Diuretiques:	Amiloride, Hydrochlorothiazide
Infertility:	Clomifene
Laxatifs:	Belladone
Neuroleptic:	Chlorpromazine, Clonazepam, Haloperidol