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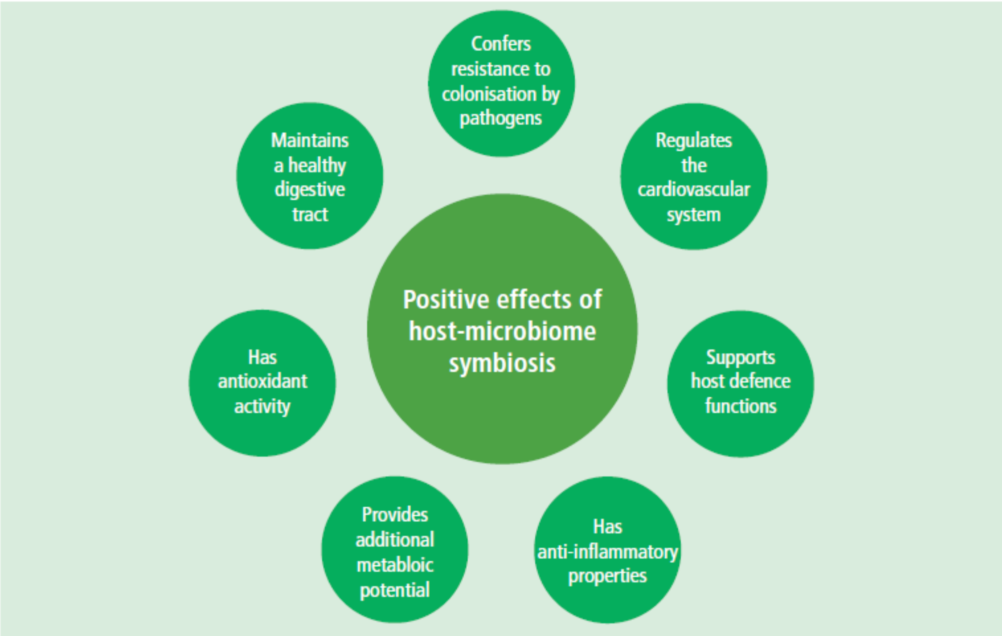
內文：

Aims :

To give an update on our current knowledge of the oral microbiome in health and disease and to discuss implications for modern-day oral healthcare

Fig. 1 Positive effects of host-microbiome symbiosis



Introduction :

The mouth is one of the most heavily colonised parts of our bodies. The purpose of this article is to update oral healthcare practitioners on our current knowledge of the oral microbiome in health and disease, to review how molecular methods of microbial characterisation have advanced our understanding, and to discuss potential implications for clinical practice

The oral microbiome: discussion

Our current understanding of the biological evolution of the microbiome

1. There is evidence that resident microbes have been performing metabolic functions in animals for at least 500 million years
2. Coevolution is documented by the many similarities in the composition and organisation of the human microbiome to that of other mammals.

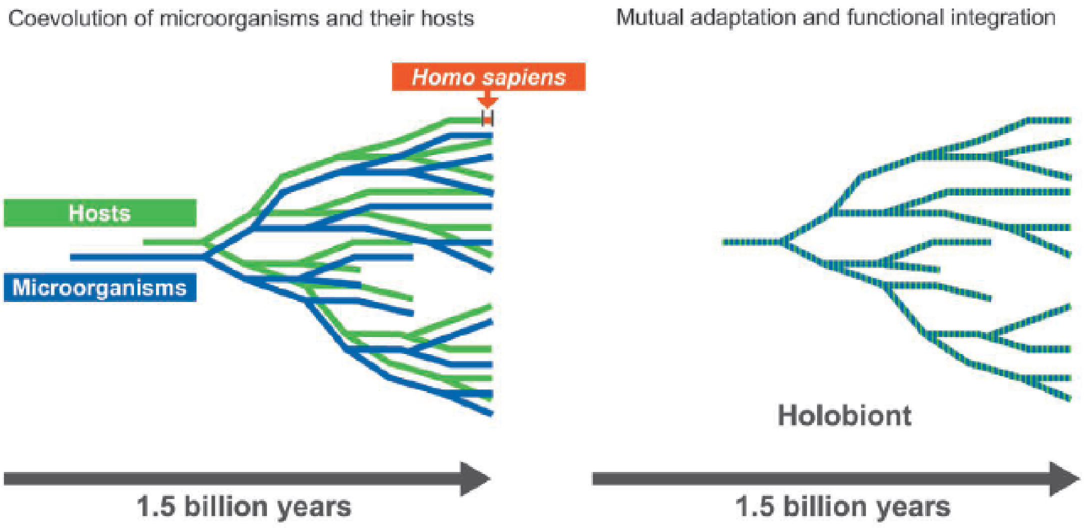


Fig. 2 History of the relationship between microorganisms and humans: The coevolution between microorganisms (blue lines) and their respective hosts (green lines) over a period of 1.5 billion years has resulted in mutual adaptation and functional integration as reflected in our own relationship to most of the microorganisms that colonise our body surfaces (*Homo sapiens*, red line)

Temporal changes in the oral microbiome

1. Introduction of refined sugar to our diet in the early times of agriculture caused certain oral bacteria to genetically evolve their metabolism to adapt to 'post-agricultural' changes in our diet
2. In addition, since the industrial revolution, humans have been more frequently exposed to agents such as heavy metals, disinfectants, biocides and antibiotics that have the potential to eradicate or debilitate many microorganisms, while positively selecting for those microbes that carry resistance determinants
3. The modern-day excessive consumption of acidic drinks and refined sugar or cigarette smoking has further impacted on the oral ecosystem, leading to diseases such as caries and periodontal disease

The human microbiome is a complex ecological community

The endogenous human microbial communities contribute to critical metabolic, physiological and immunological functions, including:

- Differentiation and maturation of the host mucosa and its immune system
- Food digestion and nutrition
- Energy generation
- Metabolic regulation and control of fat storage
- Processing and detoxification of environmental chemicals
- Maintenance of skin and mucosa barrier function
- Development and regulation of the immune system and fine-tuning of its reaction pattern, that is, the balance between pro-inflammatory and anti-inflammatory processes
- Prevention of invasion and growth of disease-promoting microorganisms (colonisation resistance).

Special considerations for the oral microbiome

1. The mouth is not a homogeneous environment for the resident microbiota, but offers several distinct habitats for microbial colonisation, such as teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, and hard and soft palate.
2. The warm and moist environment in the mouth suits the growth of many

microorganisms and offers host-derived nutrients, such as saliva proteins, glycoproteins and gingival crevicular fluid (GCF)

3. The teeth are the only natural non-shedding surfaces in the human body and provide unique opportunities for extensive biofilm formation, and a secure haven for microbial persistence
4. Dental restorations, crown and bridgework, removable prostheses and implants constitute additional non-shedding surfaces in the mouth that can influence biofilm formation and composition
5. A detailed study of 9 oral sites in 26 subjects using DNA sequencing revealed a mean of 296 species-level taxa in each individual, while 557 taxa were found among the 26 subjects

Acquisition of a normal oral microbiome

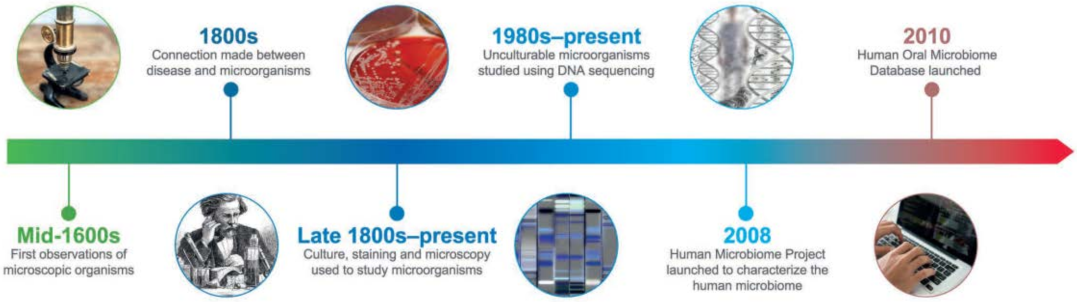
1. During birth, the mother transmits microbes to the child, and delivery mode (vaginal versus caesarean) is therefore a determinant for the type of microorganisms that a child is initially exposed to
2. Vaginally-born children showing a higher number of taxa 3 months after birth compared with children born by caesarean section
3. The method of feeding also has an effect, with 3-month-old breast-fed infants showing a higher colonisation with oral lactobacilli than formula-fed infants
4. By the age of three, the oral microbiome of children is already complex, and becomes increasingly so with age

Maintaining a healthy oral microbiome

1. Due to the interplay of the host's immune system with its microbial symbionts, acute infections of the oral mucosa are rather rare, despite dense microbial colonisation
2. Both saliva and GCF provide nutrients for microbial growth and contain components with antimicrobial activities
3. In addition to facilitating mastication, swallowing and speech, and aiding digestion, saliva contains vital enzymes and proteins that help maintain a balanced microbiota
4. Up to 108 microorganisms have been detected per millilitre of saliva, mostly derived from oral mucosal surfaces such as the tongue
5. Salivary components are the primary nutritional source for microorganisms, and are required for the development of a balanced microbiome
6. A large number of salivary components, including secretory immunoglobulin A, lactoferrin, lactoperoxidase, lysozyme, statherin and histatins, directly and indirectly regulate the microbiome, keeping it in balance
7. For example, lactoperoxidase catalyses the production of hypothiocyanite from hydrogen peroxide – a product of bacterial metabolism – and saliva-secreted thiocyanate. Hypothiocyanite exerts direct antimicrobial effects by inhibiting bacterial glycolysis
8. Another salivary component with antimicrobial potential is nitrite, converted from dietary nitrates by oral bacteria. Nitrite is further reduced to nitric oxide that can inhibit growth of cariogenic bacteria and thus may help to protect against caries
9. Proteins, including enzymes, lipids and other components (carbohydrates, nucleic acids), mainly from saliva, but also derived from GCF, the oral mucosa and bacteria, form the acquired pellicle, which modulates attachment of bacteria to dental and epithelial surfaces and protects the tooth surfaces against acid

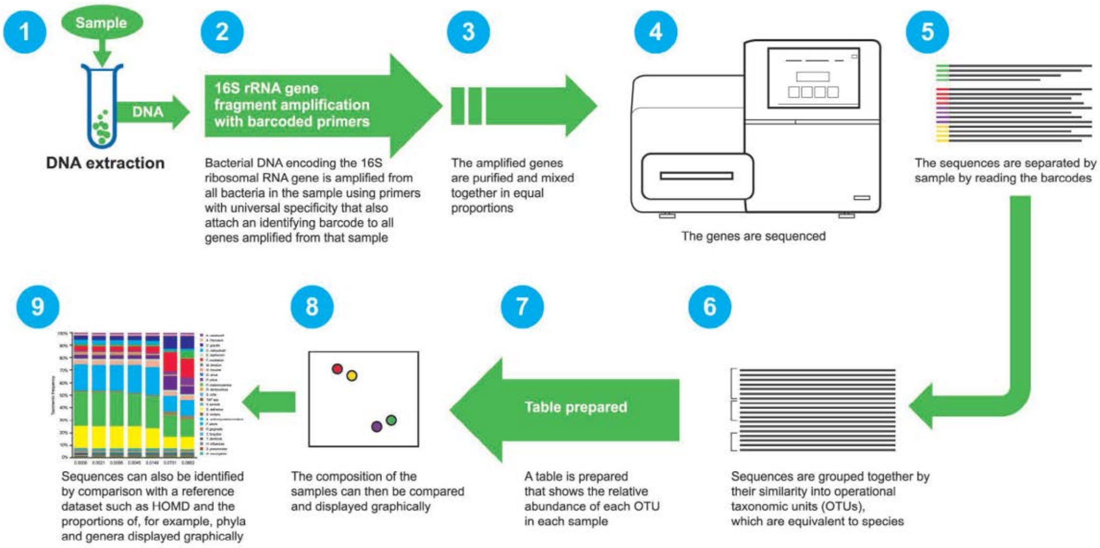
attacks

10. Saliva not only helps to maintain an environment that allows biofilms to flourish, but also modulates the layers of plaque with the help of numerous proteins, including enzymes and glycoproteins, and minerals, which control biofilm build-up and activity.



Characterisation of the oral microbiome

1. A variety of conventional methods have been used to analyse the composition of the oral microbiome, including microscopy, cultural analysis, enzymatic assays and immunoassays
2. The advent of culture-independent methods has greatly improved the detection of microorganisms, many of which cannot yet be grown in culture
3. The most common culture-independent technique to analyse the microbiome is based on 16S ribosomal RNA (16S rRNA) gene community profiling.(An RNA molecule (approximately 1500 nucleotides long) that is part of the small subunit of the ribosome in prokaryotes. 16S rRNA has both highly conserved regions, common to all prokaryotes, and hypervariable regions that are unique to particular species)
4. The 16S rRNA gene is present in all prokaryotes and contains variable regions that are unique between microorganisms and that can be used as a means of identification.
5. The traditional method of 16S rRNA gene sequencing was costly, laborious and time-consuming.Next-generation sequencing



Overview of the next-generation sequencing pipeline

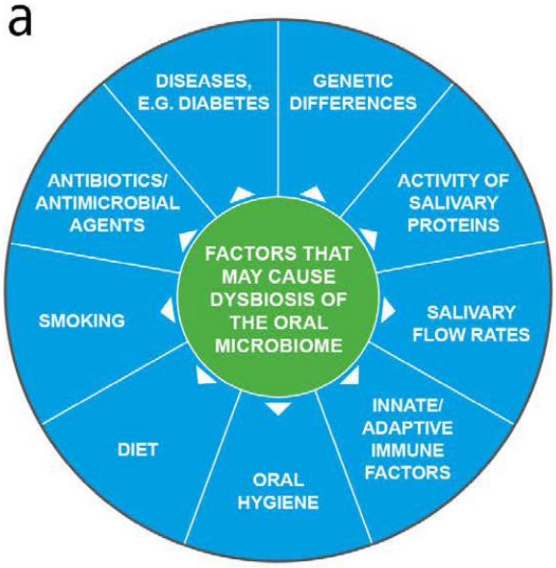
6. NGS is a useful tool that allows for high-volume studies of the genetic material in samples and has greatly increased our knowledge and understanding of the oral microbiome

7. Other modern approaches include metagenomics (Analysis of the genetic information of a complex population, typically from microbes in an environmental or host sample. The metagenome consists of the genomes of many individual microbes) and metatranscriptomics (Analysis of the active genes and species of the microbiome)
8. There is an ongoing quest to develop new methods for growing currently ‘uncultivatable’ microorganisms. Progress in this area has been made, and a new method using siderophores (small, high-affinity iron-chelating compounds secreted by microorganisms such as bacteria, fungi and grasses) has been developed to isolate and grow novel strains

An unbalanced oral microbiome may be detrimental to general health

1. The oral microbiota contributes to oral and general well-being, and its loss can be detrimental to the health of the individual
2. An example of this is the effect of nitrate-reductase-expressing oral bacteria, which have been shown to catalyse the conversion of dietary nitrates to nitrite
3. After being swallowed, salivary nitrite is further converted to nitric oxide, a potent vasodilator with antimicrobial activity which plays a critical role in sustaining cardiovascular health
4. Nitrite also stimulates gastric mucus production
5. A modest consumption of nitrate has been found to lead to a reduction of blood pressure, inhibition of platelet function and reduced endothelial dysfunction
6. A recent study has found that sustained ingestion of dietary nitrates improves vascular function in patients with hypercholesterolaemia
7. However, while dietary nitrates are able to boost nitrite formation, it is also the case that nitric oxide in tissues can interact with superoxide radicals released by immune cells to form peroxynitrite anions, which have been suggested to have detrimental actions on cells, such as DNA damage
8. Several small-scale studies have shown that the use of chlorhexidine-containing mouthwashes can reduce the concentration of nitrites in both saliva and plasma and lead to a small rise in blood pressure

Dysbiosis: the oral microbiome in disease

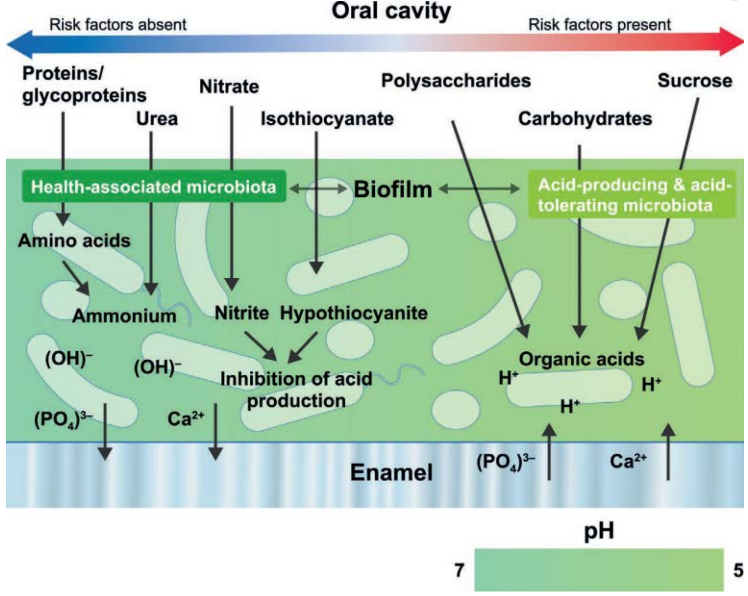


1. These include physiological changes, for example, age, or hormonal changes in puberty and pregnancy, to which healthy individuals can often adapt without detriment to their oral health

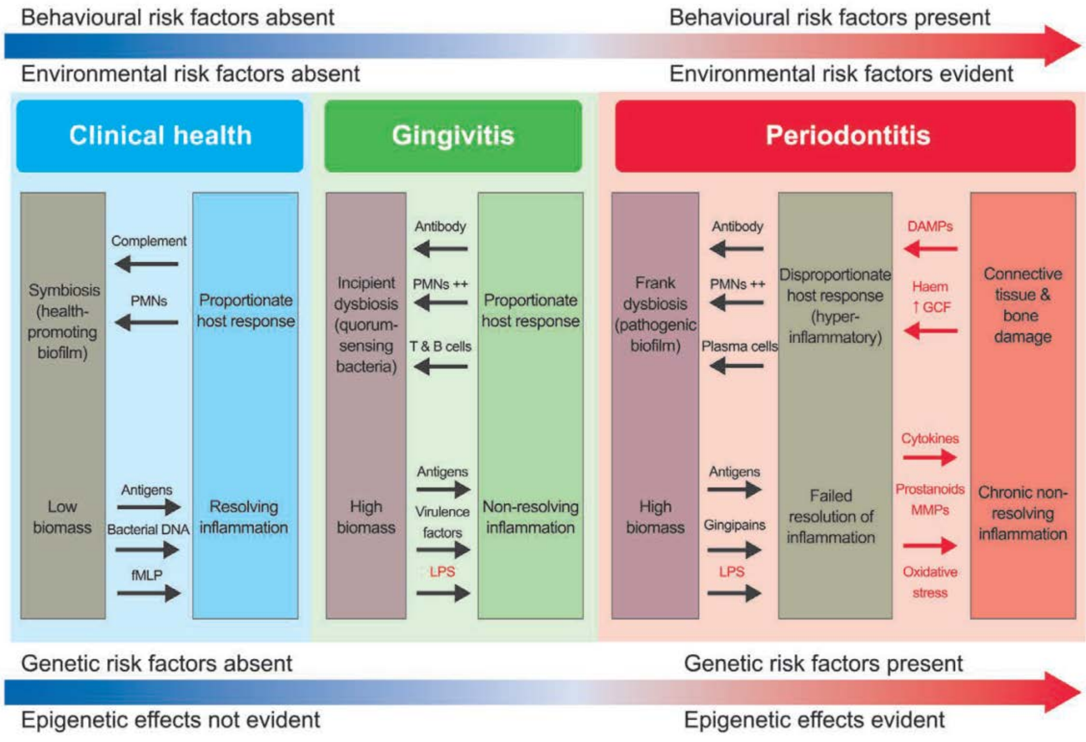
2. Modifiable factors driving oral dysbiosis include salivary gland dysfunction, poor oral hygiene, gingival inflammation and lifestyle choices, including dietary habits and smoking

Dysbiosis as origin of caries and periodontitis

1. The ‘nonspecific plaque hypothesis’ (NSPH) has its origins in the nineteenth century, but was further refined over the next hundred years. Initially, the NSPH speculated that dental infections were caused by the nonspecific over-growth of all bacteria in dental plaque
2. The observation that kanamycin was particularly effective against caries-associated species, such as streptococci, led to the emergence of the ‘specific plaque hypothesis’, which proposed that only a few species in the oral microbiome are involved in the disease process, and that targeting these species with antibiotics could cure or prevent disease (initially caries and later periodontitis)
3. This hypothesis introduces the concept that disease can be prevented not only by directly inhibiting the putative pathogens, but also by interfering with the environmental factors driving the selection and enrichment of these bacteria
4. For instance, in caries, an increased frequency of sugar intake, or a reduction in saliva flow, results in plaque biofilms that are exposed for longer and more regular periods to lower pH levels. This selects for organisms that produce acids themselves and/or are more tolerant of an acidic environment, at the expense of bacteria that thrive in neutral conditions or contribute to pH neutralisation



5. Accumulation of triggers gingivitis; however, the presence of biofilm alone is not sufficient to advance into periodontitis.



Chapple 2015

6. A dysbiotic microbial community subverts the host response so that most tissue damage is due to an inappropriate and uncontrolled level of inflammation.
7. Local inflammation resulting from biofilm accumulation causes an increased flow of the nutrient-rich GCF and potentially bleeding, whereby the site becomes deprived of oxygen, favouring the growth of anaerobic microbes
8. The inflammatory changes in the periodontal environment provide an ideal environment for the growth of obligatory anaerobic and protein-dependent bacteria that reside in the gingival crevice, driving a shift from a symbiotic microbiome to dysbiosis
9. Inflammation-induced micro-ulceration of the sulcular epithelium results in the leakage of blood (and thereby iron) into the gingival crevice. The resulting conditions are conducive for periodontitis-associated species such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans to survive and flourish
10. The dysbiotic microbiota induces the destruction of the periodontal tissue by a dysregulated host inflammatory immune response, which in turn provides new tissue breakdown-derived nutrients for the bacteria
11. Through evolution, bacteria have adapted to take advantage of inflammation-derived nutrients, promoting further dysbiosis and tissue disruption, creating a self-perpetuating pathogenic cycle
12. This is even more aggravated in diabetes, which is associated with metabolic dysregulation that modulates the inflammatory response, leading to a highly inflammatory state that further accelerates tissue destruction and attachment loss.

Systemic consequences of oral dysbiosis

1. Oral bacteria have been proposed to play a role in a number of systemic diseases, including cardiovascular disease, rheumatoid arthritis, adverse pregnancy outcomes, stroke, inflammatory bowel disease and colorectal cancer, respiratory tract infection, meningitis or brain abscesses, lung, liver or splenic abscesses, appendicitis, pneumonia and diabetes

2. It is well established that severe periodontitis adversely affects glycaemic control in diabetes and glycaemia in subjects who do not have diabetes
3. Severe periodontitis poses an increased risk for the onset of type 2 diabetes, and there is a direct and dose-dependent relationship between the severity of periodontitis and diabetic complications

Conclusions

Improved understanding of the holobiont – current and future implications for clinical practice

1. Thus, it is pivotal that both patients and healthcare professionals embrace the concept of a balanced oral microbiome and its importance in oral and systemic health
2. Treatment sessions should include prevention strategies, such as advice on oral hygiene practices and on diet and smoking
3. Oral healthcare professionals can achieve this by educating patients on appropriate lifestyle choices and the application of effective plaque control techniques that preserve dental biofilms at levels compatible with oral health
4. Indiscriminate use of antibiotics for the treatment of oral diseases should be avoided, aiming to safeguard the beneficial oral microbiota and avoid antibiotic resistance
5. For the control of caries, in addition to use of topical fluoride, measures should be taken that encourage a shift away from an acidic environment, through reduction in the amount and frequency of the consumption of sucrose and acidic drinks (even if the latter are sugar-free), together with supplementation with agents that can reduce acid production and/or promote alkali generation within dental plaque
6. For periodontal disease, treatment strategies should aim for mechanical reduction of accumulated biofilm by mechanical removal of plaque back to levels compatible with oral health
7. This would reduce inflammation and the accompanying flow of GCF, and promote a favourable microenvironment to support formation of a balanced microbiome. Where relevant, patients should also be advised on smoking cessation and control of diabetes

題號	**題目**
1	Hemin iron from the breakdown of host hemoglobin may be important in the metabolism of which species? **(A)** *Tannerella forsythensis* **(B)** *Prevotella intermedia* **(C)** *Porphyromonas gingivalis* **(D)** *Fusobacterium nucleatum*
答案(C)	**出處**：*Carranza's* Clinical Periodontology 12th Edition CHAPTER 8 Biofilm and Periodontal Microbiology P.149
題號	**題目**
2	The amount of GCF is greater when inflammation is present, and it is sometimes proportional to the severity of inflammation. GCF production is not increased by **(A)** trauma from occlusion **(B)** Sex Hormones **(C)** Smoking. **(D)** Periodontal Therapy

答案(A)	**出處**：*Carranza's* Clinical Periodontology 12th Edition CHAPTER 13 Defense Mechanisms of the Gingiva p.216