Research Review Speaker Series™

Plaque Biofilm Management: Restorative Microbiology and Tailored Comprehensive Oral Care

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This publication is a summary of a recent presentation by Professor John G Thomas MS, PhD, HCLD, delivered to hygienists and dentists in Australia and New Zealand.

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Professor Thomas is presently director of the Biofilm Research Laboratory for Translational Studies in Medicine, Dentistry and Industry at West Virginia University.

He created the International Tri-University Biofilm Research Consortium in 2002 to further global investigations and education (www.hsc.wvu.edu/som/pathology/thomas) and has published extensively on diverse microbial topics.

Professor Thomas integrates four academic and educational positions at West Virginia University: School of Medicine, Pathology, School of Dentistry, Periodontology, School of Pharmacy, and Graduate School, Department of Microbiology and Cell Biology. He also holds teaching positions at Rutgers University and internationally is a Visiting Professor (Honorary) at Cardiff University, School of Dental Medicine and National University of Singapore, School of Dentistry.

In 2008, Professor Thomas was invited to join the Scientific Advisory Council, American Dental Association, and in 2007, he was given the highest achievement award for University Teaching at WVU, recognised by students. In 2009, Professor Thomas was awarded the Student Research Development Award by the School of Dentistry, having received the award in 2006 and 1998.

The oral flora is the most complex, diverse and integrated microbiota of Homo sapiens with more than 1,000 phylotypes called Operational Taxonomic Units. This Oral Microbial Signature is distinct for each person, changing with age, and it may also be a possible predictor of health or disease, oral and systemic.

Gingivitis, periodontitis and endodontic disease are often associated with an unbalanced oral biofilm bioburden. Disease mediation is both microbial bioburden and inflammatory associated, while pathogenicity is linked to 'colonisation resistance'.

This presentation assessed the consequences of an unbalanced oral microbial environment with consideration to the changes from birth to death as a risk factor for the maintenance of health. As health care professionals, in the developed world, we are living in an age of chronic condition management. Understanding the role of an unbalanced oral biofilm bioburden in the disease mediation process of gingivitis, periodontitis and endodontic inflammation provides the health care professional with valuable pathways of care for patients – in preventing disease, maintaining health and maximising the effectiveness of restorative procedures.

The presentation also explored the role of probiotics in conjunction with the daily application of mechanical biofilm removal, chemical biofilm control and the utilisation of anti-infectives.

50 years ago – where to now?

Professor Thomas proposed to the audience that oral disease is still the most unrecognised number one potentially infectious state of all medical microbiology. Despite the advances made with dental tools and the grouping of microbials, the approach to dentistry is essentially unchanged from over 50 years ago. The literature informs us that we have identified only 5% of the oral flora. Caries and periodontal disease still plague us. Why is this so? With the recognition that every person is a walking microbial organism living in a microbial world comes the realisation that we must learn to live with microbes in a friendly environment, which is dramatically changing the medical management of patients.

Professor Thomas further shared his thoughts on the past, present and future thinking related to disease prevention and management by health professionals.

Biofilms

Oral care may be conceptualised as airway management, as there is no 'trap door' separating the head from the rest of the body. Whereas in the 1880s Robert Koch's view was one microbe – one disease, it is now known that biofilm is made up of multiple species. If we see biofilm as the pre-eminent model, then almost every infectious disease of today affecting both hospitalised and non-hospitalised patients may be caused by exactly the same organisation.

No site of any microbial disease process contains only one microbe of only one type. Notably, organisms have two life forms – the planktonic (free floating) and the sessile (attached) or biofilm form. Indeed, saliva with planktonic organisms is a vehicle by which continual inoculation of the site will occur as every microbe that is planktonic has the capacity to attach. Microbes choose to attach and do so very quickly, within about 28 seconds. Thus, microbe management should not be focusing on whichever microbe has to be resolved, but rather, whether it is attached or free floating.

The disease process occurs when the amount of biofilm exceeds that of planktonic organisms. An appreciation of the ratio between planktonic and biofilm form helps to determine best patient care management.[1]

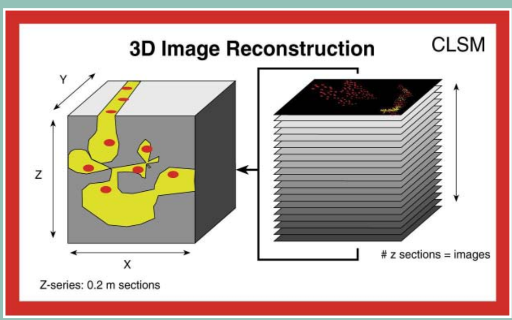
Dental plaque: a microbial biofilm

The biofilm is recognised as being a structured environment of multiple organisms, living in an incredibly well organised community. For instance, micrograph review of tissue specimens taken from ethmoid and maxillary sinuses of patients with chronic rhinosinusitis have revealed biofilm findings such as water channels, 3-D structure, and matrix-embedding spherical or elliptical bodies within the size range of bacterial organisms 0.05 µm to 5.0 µm.[2]

The development of the dental plaque biofilm colonised by only *Streptococcus mutans* is referred to as a mono-species biofilm. If after 8 seconds to 8 hours it begins to form a co-biofilm with other organisms, it then becomes a multi-species biofilm. Notably, every biofilm community on the tooth's surface forms a separate silo. Every part of the biofilm has a resilience factor. Medicine can cure one-microbe biofilms, but co-biofilms are problematic. In the worst case scenario, many silos are all working independently yet together, as a resilient ecosystem.[3]

Within the dental plaque biofilm structure, those organisms attached at the lowest point are quite dense and rod-shaped; the spacing and organisation becomes more detailed and also more open, as the organisms move up towards the top of the structure. Initial biofilms are usually cocci, then rods. Whereas they are basically inert at the bottom of the structure, dental plaque biofilms evolve into more of a spore-like substance at the top of the structure The idea of complex structures and channels running throughout the plaque has implications for penetration efficacy of antimicrobial treatments and mechanical removal.[3,4]

Biofilm 3-D Structure



Optical sectioning (X, Y, Z)

Poloxamer F-127

- Co-polymer "poloxyethylene/polyoxypropylene
- thermo-reversible gelation
- acts as a "scaffold", supporting bacteria in 3 dimensions
- biofilm formation due to:
  close location of bacteria, co-aggregation, high densities

Professor Thomas demonstrated these challenges via his unique 3-D images of the dental plaque biofilm from Stage I to Stage IV. The images were captured utilising optical sectioning technology of a dental plaque biofilm specimen. The specimen is captured in a thermo-reversible gelation that acts as a scaffold to support the bacteria in 3 dimensions. The major advantage of this material is that it is clear; it can be optically sectioned and analysed within 12 minutes. This information becomes a valuable tool for practitioners wanting to provide options to maximise and maintain health outcomes for their patients. It is possible to identify the microbes, the organisation of the plaque, the population density and what can be done differently. For example, when recommending an antiseptic mouthrinse, the molecular size of the active ingredient, the pH and the charge status must be considered in the context of the structure of the dental plaque biofilm.

Chlorhexidine is cationic (positively charged) and has a molecular weight of 505.45. Ability to penetrate the negatively-charged dental plaque biofilm and the possible interaction with other ions (e.g. fluoride) raises questions for use in controlling bacteria. Conversely, essential oils such as Eucalyptol, Thymol, Methyl Salicylate and Menthol have a molecular weight range of 140–156 and are neutrally charged (anionic).

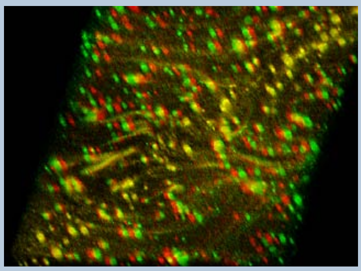
Understanding the status of biofilm development via this unique examination technology supports treatment options and recommendations for daily home care. While the nuances of the dental plaque biofilm are specific to each individual, understanding the key properties of a biofilm is critical to care.

A Biotic Substratum

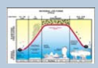
In vitro analyses of gram positive organism B using Poloxamer Stage I (microcolonies); animation

Anaglyph

Quantification
Biofilm Thickness
Biovolume
Substratum Coverage



Microcolony
Organization pattern
Spatial Arrangement
Complexity Heterogen
Biomass
Thickness 250 µm



Images provided by Convatec
Dr S Percival & Bowers

Key properties of a biofilm

Professor Thomas proposed that the biofilm has almost nothing to do with microbiology – it is essentially a hydrated polymer. Scaling and root planing via the deposition of energy from an ultrasonic or hand instruments will not totally eliminate a biofilm. Professor Thomas believes this approach alone is somewhat 'anti-dental'.

Biofilm susceptibility towards biocides and mechanical removal may be dependent on the maturity of the structure:

- *In vitro* analyses of abiotic substratum reveal that Stage I (microcolonies of monospecies) is very early in the life of a biofilm. The penetration of an antiseptic mouth rinse will work, because at this stage the biofilm is not very stable. Plaque can be managed very early in the disease process by traditional means.
- However, after 8 hours, the structure is now a co-biofilm, with more than 3 species co-existing, all sharing the same pH range. Think about the management potential in regard to checking the pH of a biofilm.
- After 36 hours, the biofilm has a huge organisation and is highly complex. There is evidence of stable integration of function and increased bio-diversity with focused marker organisms. There is also evidence of cross-talk between the species.[3] By Stage IV, the plaque biofilm has become very interesting, whereby a syringe can be used to extract certain layers of the microbe pool. We have yet to identify all of the flora in this organism pool. When these organisms die or begin to break up, where do they go? What kinds of things attach to certain surfaces that a patient uses, such as a toothbrush? Professor Thomas believes that in the next 3 years, knowledge of dental plaque biofilms will increase rapidly as science and technology offer greater insight to this complex world.
- Stage IV is very important in terms of transmission. When the organisms leave this stage, they become planktonic (free floating) and may re-colonise in a different location. What niche in the oral cavity will they occupy?

The first step in biofilm development is the covering of the teeth with an amorphous organic film called the 'pellicle', which is mainly a glycoprotein from saliva. Dental pellicle protects the tooth by interfering with bacterial adhesion, affecting mineral balance and assisting colonisation by bacteria. Thus, biofilms are not entirely bad.

The dental plaque biofilm generally, as assessed by molecular methods, comprises multiple species. Which one should be treated? *Streptococcus mutans*? That would be appropriate, for controlling acid, decalcification, and all the reasons associated with the risk of developing caries. What about the impact of other organisms in the dental plaque biofilm?

Candida albicans: The Universal Co-Aggregate

The emergence of *Candida albicans* as a major contributor to the initiation of caries development necessitates an even greater understanding of the complexities of the oral flora. Firstly, Candida is a bacterial-like organism, a eukaryote (without cell wall material), which mimics the patient's own cell structure and grows in two styles. Organisms can attach to it. Importantly, patients who have Candida in a biofilm have reduced resilience by over 1000-fold to most known therapies. This is a huge potential problem. Notably, *Candida albicans* exists in healthy normal individuals, who have been identified as carrying between 9 and 27 different species of Candida.[5] Together with *S. mutans*, *Candida albicans* produces a major acid shift that contributes to the risk of developing dental caries.

In the diversity of procaryotic biofilms, *Candida albicans* is the key biofilm building block and potential treatment target. In addition, it is well understood that the hallmark of an immune-suppressed patient is the presence of *Candida albicans* or thrush, which is often found on the tongue and/or palate. How should this be treated and with what product? While the unwanted organisms have to be reduced, the oral flora must not be eradicated totally. This is the worst form of action to take and the subsequent imbalance of oral flora may create an environment of microbial resistance to accepted oral care recommendations. Therefore, as clinicians, we need to consider the impact of recommended care beyond resolving the most obvious and immediate problem.

Oral Probiotics

Probiotics have enjoyed a higher profile and broader acceptance in the past decade as a complementary option to support healthy outcomes. In the context of oral health, more than 160 published papers have considered the role of probiotics to support improved outcomes in conjunction with recommended dental therapies. The promotion of colonisation from less pathogenic bacteria species, the effect on local immunity and modulation of systemic immune function are all mechanisms of action from oral probiotics. Utilisation of oral probiotics have been part of the accepted treatment protocols in European countries such as Sweden, Spain, Denmark and the UK together with the US and Japan. Professor Thomas is extensively involved in the further development of next generation probiotics and validation of efficacy via clinical trials.

Professor Thomas also indicated that the use of antibiotics in hospitals that he visits is strictly controlled on a rotation basis to minimise the risk of resistance development. Very much like 'crop rotation', the natural flora is given the opportunity to re-establish a balanced environment. When the medical practitioner prescribes an antibiotic for patient care, a window appears on screen with the nominated probiotic to complement the health care regimen. These approaches to care are critical to reducing the risk of developing antibiotic resistance and irrevocably changing the natural balance of flora that is protective to the individual.

However Professor Thomas recognises the continued challenges in promoting this approach as demonstrated by guidelines in the US regarding the daily use of chlorhexidine body washes for those in long term care. He felt that this guideline is a serious risk to patient health and ability to resist microbial infection. Understanding the pathways to work with the patient's best line of defence, their natural flora, is greatly underrated and underutilised.

Professor Thomas asked the audience to consider the maintenance of oral health in the context of re-establishing normal flora for both clinical intervention and home care professional recommendation. From the first intervention of clinical treatment, the biofilm is an important consideration. What do we choose as a pre-procedural rinse? Will our choice be effective in reducing the risk of aerosol contamination? How will the patient manage or react to our choice of pre-procedural rinse? Professor Thomas and colleagues recommend Essential Oils antiseptic mouth rinse for their patients in the dental clinic and acute care hospital setting. Scaling and root planing can reduce the established bio-burden for the patient but we must understand the short and long term impact of this activity. Likewise, daily oral hygiene needs should encompass the optimal routines of tailored comprehensive oral care – brushing, flossing (or other interdental cleaning) and an antiseptic mouth rinse.

Oral Microbial Signature

Thus, dentistry is no longer simply about management of periodontal disease and restoration of dental hard tissues lost to caries. Rather, it is about defining potential risk of disease as determined by the oral flora of the individual. The Oral Microbial Signature (OMS) is a term coined by Professor Thomas to capture the unique identification of the individual that is afforded by the oral flora. Indeed, according to the Federal Bureau of Investigation (FBI), the single most predictive identification value of an individual is a person's oral flora; not the thumbprint, voice box or retina. The FBI continues to support extensive studies to understand more about the oral flora. We also have NIH-funded footprints or roadmaps to study the genes of all the microbes of not only the oral cavity but the host. These will cross-link all the microbiology genetic information with all the disease genetic information to obtain markers and predictors.[6] In the near future, will we be able to use our iPhones with our patients as a way of mapping their oral flora and co-morbidities, and create a management style and risk assessment? The possibilities are quite real as our world is changing at a rapid rate. Already we have seen the measurement of tumour markers in saliva being introduced to the diagnostic screening process. So far, 16 tumour markers have been identified. The world will become more complex, more demanding and more requiring of information. What does this all mean?

Tailored Comprehensive Oral Care

Professor Thomas favours 'minimal intervention' and encourages clinicians to avoid changing the biofilm that is a protective environment. He questions whether we are still doing things because someone taught us to do that as a student, or whether these actions are backed by current literature support. Professor Thomas and colleagues teach their students, hygienists and dentists that microbiology should be considered in the context of life from birth to death. Our role is to be stewards of the oral microbial flora, not to just treat the event that the patient consulted on, because that may present some problems in the future. For example, everyone at some time is going to be in a supported aged care facility and they will have oral flora. Any resistance that is set up in our management has the potential to show up in 10 years' time as an issue. Clinicians have to realise they are not dealing with a patient only today – but throughout their lifetime. Furthermore, it is not only oral disease, but systemic disease as well. Professor Thomas estimates that there are at least nine systemic diseases where good oral hygiene can greatly assist quality of life.[7] In hospitals that Professor Thomas visits, 80% of ICU patients have a biofilm disease. The most defining fact in ventilator-associated pneumonia (VAP) is oral flora.[8] How are we providing tailored comprehensive oral care for these patients? Does the dental team contribute to supporting a balanced oral flora to assist in avoiding the potential ramifications of VAP? What is the difference between a chronic non-healing wound (e.g. a toothless periodontal pocket) and a deep-seated wound? We now know that all the structures that dentists deal with are the same as the structures of a wound site of the skin. Wounds in stages I and II can be managed as usual. For those that are in stage IV, where is the microbe going? These questions will become more and more critical as the approach to biofilm management becomes a focal point of health care.

Take home messages

We are just beginning to unravel old methods and changing the nature of things. A tool that will be increasingly talked about in the literature is critical colonisation – Stage III – a well-formed biofilm.

The most important and unrecognised organism in the oral flora is *Candida albicans*. How do we manage it? We are just beginning to learn.

New methods are going to be molecular and these will highlight a whole new cadre of microbes.

We are told we know about 5% of microbiology. Thus, it will be a learning curve.

The human genome study has been followed by the international human microbe and the oral human micro bio roadmap. This will tell us how to match human disease from human genomes with bacterial disease from biofilms and integrate them. This is expected to occur within the next 3–5 years.

Lastly, the US is talking about saliva-based theragnostics – therapy and diagnosis from a single salivary specimen.

THE MICRO MINI SERIES

Below is a series of 30-minute lectures on biofilms and their importance in today's medicine:

1. Biofilms: Architect of Survival
2. Biofilms: Architect of Chronic Diseases Head to Toe
3. A Comprehensive Engineered Ventilator-Endotrach-Lung (VEL) Model
4. Diagnostic Microbiology, Pathology and Biofilms
5. Rediscovering the Benefit of "Microbial Engineering": Probiotics
6. Putting the Pieces Together: The Link Between Oral Care, Biofilms, and Pneumonia
7. Defining the Impact of Microbes in Chronic Wounds: A Biofilm Reactor
8. Prebiotics & Probiotics: Old Fad with New Interventions – NEW
9. A Biofilms Procaryotic Tumors? Or... Are Tumors Eucaryotic Biofilms? – NEW
10. Universal Microbial Principles. Linking the Old and the New – NEW

For more information on the Micro Mini Series visit:
http://www.hsc.wvu.edu/som/Pathology/Thomas/Educational-Resources/TheMicroMiniSeries.aspx

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