Research Review Speaker Series™

'Pay me now or pay me later': the lifelong consequences of oral flora imbalance

Making Education Easy

Professor John Thomas MS, PhD, HCLD

Professor Thomas is the former 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.

During his former long tenure at West Virginia University, Professor Thomas integrated four academic and educational positions at: School of Medicine, Pathology, School of Dentistry, Periodontology, School of Pharmacy, and Graduate School, Department of Microbiology and Cell Biology. He still 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.

This publication is a summary of a recent presentation by Professor John G Thomas, former Director of the Biofilm Research Laboratory for Translational Studies in Medicine, Dentistry and Industry at West Virginia University, USA. He spoke to dentists, dental hygienists, oral health therapists, special needs dentists and dental therapists in Brisbane, Sydney and Melbourne during October 2012. The key focus of the presentation was to encourage health professionals to recognise that we are all walking microbial organisms living in a microbial world and how an individual's oral flora may define potential risk of disease. Professor Thomas stressed that clinicians must be a partner to the microbial world that is their patient.

I. Introduction: Our Microbial World

1. Biofilms Made Easy: "A Picture Tutorial"
   http://www.hsc.wvu.edu/som/Pathology/Thomas/PDFS/Educational-Resources/Biofilms-For-Everyone-Booklet.pdf
2. Mini-Micro Series
   http://www.hsc.wvu.edu/som/Pathology/Thomas/Educational-Resources/TheMicroMiniSeries.aspx
3. Educational Resources
   http://www.hsc.wvu.edu/som/Pathology/Thomas/Educational-Resources/
4. Case Studies
   http://www.hsc.wvu.edu/som/Pathology/Thomas/PDFS/Educational-Resources/Oral-Infections-AntiInfective-Mngt-&-Cases.pdf
5. Post-Graduate Dental Education (Under Construction)
   http://www.hsc.wvu.edu/som/Pathology/Thomas/Online-Courses/

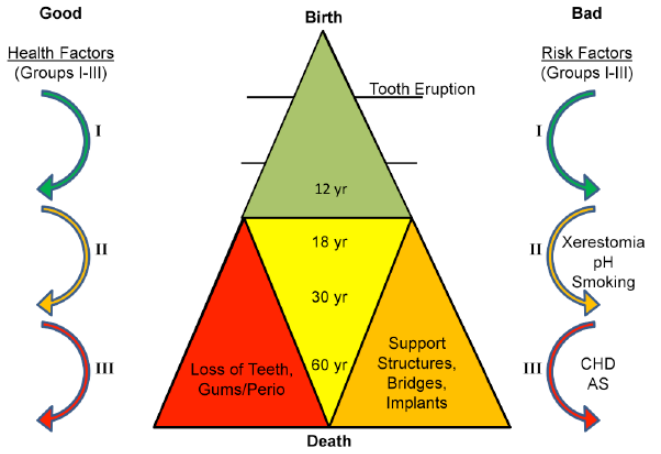
Microbiology, health and risk of disease should be considered in a context of the full lifespan, with comprehensive oral care (COC) maintaining a continuum from birth to death. As we begin to understand more about disease risk throughout the life span starting with the NICU/paediatric ICU, progressing through the adult ICU, the step-down unit, rehabilitation, assisted living, long-term outpatient care, nursing home, hospice then death, it becomes more apparent that if we fail to partner with the microbes that cohabitate with the individual, we will lose the war. We cannot eliminate bugs – we have to work with them.

Prof Thomas stressed that diversity of the microbial world is most extensive firstly in the oral cavity and secondly in the colon. How do these two important reservoirs interact? In his experience as an educator, Professor Thomas often finds that students of oral health are focused on everything from the neck up. He has also found that medical students are focused on everything from the neck down. Is there a trap door between the head and the rest of the body? Do we as oral health professionals ever consider the colon? How many times will the medical team talk to the dental team? Communication between health professionals to support improved patient outcomes is an area of critical opportunity. Professor Thomas shared the example of caring for a patient who is intubated in the ICU. The risk of pneumonia is increased by 1500-fold in patients on a mechanical ventilator if they enter the intensive care unit (ICU) with poor oral hygiene. Who in the hospital

Watch the video highlights of **Professor Thomas's talk** at www.researchreview.com.au/videothomas

makes an assessment of the oral care of a patient at the time of intubation? The anaesthesiologist, but what does an anaesthesiologist know about oral health? Nevertheless, this is the standard of care. Who cleans the mouth of a patient when undergoing intubation? The cost of providing care for a patient who has acquired Ventilator Associated Pneumonia (VAP) impacts significantly on an already overburdened health system. VAP is largely preventable if oral health is maintained. Unfortunately, in West Virginia from the experience of Professor Thomas, sixty percent of ICU patients who undergo mechanical ventilation and intubation come directly from nursing homes, where no dental care is provided. Conversely, how many NICU babies are on ventilators? What do we know about oral health and the pneumonia of little babies? Nothing. Who takes care of the oral health of a newborn? Yet evidence clearly shows that this aspect is so germane in the disease process that dental health professionals must be involved.

Therefore, the integration of medical/dental holistic care is vital to support optimal health outcomes for all stages of life.



CHD = chronic heart disease; AS = Arterial sclerosis

Figure 1. 'Risk factors' impacting tailored comprehensive oral care: the oral microbial tree of life and risk factors.

II. Metagenomics, Microbiota, Mycobiota: Your Patient

As the scientific community continues to understand more about the microbial world that we all inhabit, new terminology is developed to accurately reflect the most up to date knowledge. Dr. Robert Koch's view, formulated in the 1880s, has long informed the thinking that there is one bug and one disease. However, we now know that disease consists of multiple organisms living as a biofilm; in essence, nothing that we deal with today is just one bug. Throughout our careers as health professionals, we need to redefine ourselves with what tools are available to us. One of the more recent scientific investigations, the human genome project, has supported a more comprehensive understanding of the individuals' bacteria signatures.

The initial human genome roadmap studies into the microbial world focused on just bacteria. The US and European research into the human microbiome yielded substantial information, but it was limited by insufficient information on the *mycobiome* or yeast/fungi component. This has since been addressed and we now know that individuals can be defined by their genetic makeup; humans have about 10^{12} eukaryotic cells and 23,000 genes. We now know that we have more fungi and genes established with yeast than we have bacteria. It is also known that the cohabitation of microbiota (bacteria) and mycobiota (fungi) from birth results in the synthesising of around 8,000,000 genes of bacteria products every 20 minutes in the body. The library of microbial interface with humans is hugely in excess of the quantity of prokaryotic cells (10^{14}) in the body. Yet we have defined our existence by the Robert Koch/Louis Pasteur theme, which advises treatment and elimination of a microbiome instead of understanding the metagenomics of the patient. The term metagenomics describes our body as an aggregate of the genes that produce products and proteins every day (see Fig. 2).

- The earliest time of our life is when we seem to happily co-exist with our biological compatriots (bugs, bacteria, fungi)
- Between approximately ages 12 and 21 years, serious problems begin to arise, as a consequence of what our bugs eat
- As we approach the ages of 20–21 years and up to around age 50 years, serious problems arise where organisms produce disease consequences (e.g., gastroenteritis with *Clostridium difficile*, *Helicobacter pylori*)
- Upon approaching ages 65 years upwards, tumour markers appear and bugs clearly have the capacity to change our prokaryotic and eukaryotic cells into a unhealthy environment.

In each of these four stages, examinations of the mouth reveal very different environments in which that patient's health is being addressed. Disease management from birth to death is about recognising stewardship of the existing flora.

In June 2012, the literature was deluged with new references about metagenomics. The entire June issue of *Science* discussed the gut and microbiota, while *Scientific American* simultaneously published new revelations involving the intestinal ecosystem.[1,2] Notably, the US FDA has announced that the most definitive way with bioterrorism of identifying a person is not by voiceprint or iris print, but by the microbial flora.

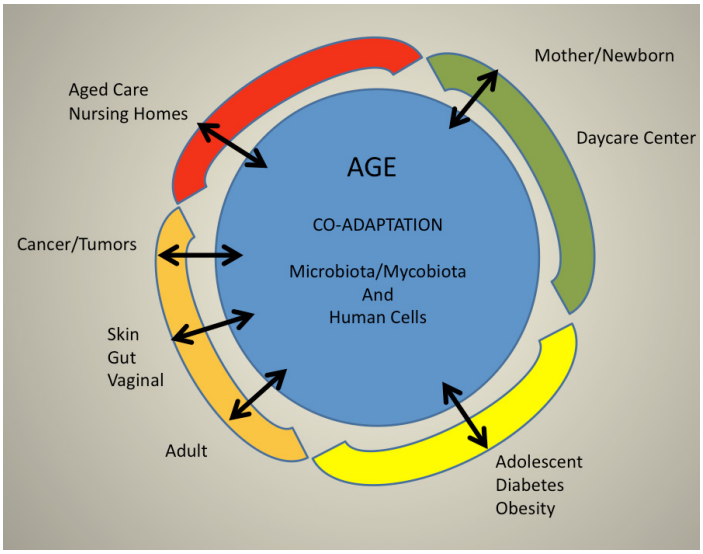


Figure 2. We live in a metagenomics microbial world.

As a greater understanding of metagenomics has emerged, training in microbiology has also evolved. Informed by the theory of disease as enunciated by Koch and Pasteur, microbiology training has traditionally addressed a hierarchical order from domains to species. Now, we have learned that we must address a phylum (i.e., actinobacteria, bacteroidetes, firmicutes, proteobacteria), because we are a co-habitat of clusters. One bug is not the issue; rather, the issue is phyla that bugs exist with and the cluster at any particular time of our lifespan.

Investigations led by the US National Institutes of Health that examined the phyla at each site in the human body have underlined a cluster concept of phyla (*we*) versus species (*I*).[3] Clusters define conditions such as obesity, dementia, and periodontal disease; these diseases are not a result of individual bugs. Clusters define the health status of an individual patient. Clinical laboratory identification of microbes in seriously ill patients (20% by culture, 80% by molecular methods) reveals clusters and yields more meaningful information for the clinicians.

Our body's clusters differ by bodily site (e.g., genital areas, skin, nostril, etc.). Furthermore, research into phyla and clusters has revealed that every individual is defined by one of three enteroptypes, as identified by their stool flora.[4] Importantly, metagenomics defines an individual's colon content by age, which means that a single patient may not respond the same to a pharmacological treatment when the enterotype differs between consultations. Prof Thomas predicts that in future, patient dental management will also depend upon the patient's enterotype. West Virginia University Hospital has already started to

do this with diabetes patients, where the enterotype makes a difference in management, based on the phyla and metagenomics of the colon. This makes sense as after all, the colon is the second largest reservoir of microbes after the mouth.

Recently, Prof Thomas and colleagues at West Virginia University investigated the potential association between oral health and cognitive function, using high throughput DNA sequencing to detect differences in the subgingival plaque microbiome in elderly persons (aged ≥70 years) with and without dementia.[5] Although this pilot study involved only 10 individuals (cognitively assessed as normal [n=2], cognitively impaired without dementia [n=3], and with dementia [n=5]), a comparison of samples between the non-dementia and the dementia groups revealed that the non-dementia samples had a consistently higher proportion of Fusobacteria-specific sequences and a lower proportion of sequences from the Bacteroidetes phylum. As this investigation continues, Professor Thomas and his colleagues will explore this relationship as a possible oral marker for disease.

III. Biofilms, Socio-microbiology

Scaling and root planing will not totally eliminate the dental plaque microbial biofilm as it is composed of tooth surface-associated micro-oganisms in complex, spatially-organised, wall-less structures with capillaries that are capable of moving fluids while developing a resistant, protective environment.

Like a tumour, a biofilm may be staged by growth. The stage of the biofilm (the maturity of the structure) has important consequences for its susceptibility towards biocides and mechanical removal:

- Stage I (microcolonies of monospecies) is loosely organised and very early in the life of a biofilm. Metalith images reveal that the plaque has spatial arrangements that allow penetration by certain sizes of molecule. Thus, choice of molecule (chlorhexidine, essential oils) in an oral health rinse is important.
- At about 72 hours, the structure is in Stage II and is now a co-biofilm, with more than 3 species co-existing. It has begun to coalesce into regions characterised by different pH ranges (5, 11, and neutral). A biofilm is a composite of community pH, going from pH 3 to pH 11. What oral rinse is stable from pH 3 to 11? What antibiotic is stable from pH 3 to 11?
- After entering Stage III, the biofilm has become a huge organisation and a highly complex environment. The biofilm is now impenetrable and is a well-defined, multi-species. It is a phylum containing 30 to 40 phyla, with up to 100 individual species.
- In Stage IV, the environment of the organisms is now so beautifully organised that they begin to metastasise.

Prof Thomas and colleagues use microbiological techniques to stage the biofilm within about 30 minutes. This information helps to predict which patients will be easily manageable or what type of interface can be used.

Microbiota and mycobiota form an arrangement within the biofilm that allows other organisms to attach to it. *Candida abicans* is the universal co-aggregate. In the diversity of prokaryotic biofilms, this biphasic eukaryote is the key biofilm building block and potential treatment target: this yeast-like structure addresses and allows attachment of multiple species, yet *C. albicans* is one of the most unrecognised oral pathogens that is not part of the treatment regimes and should be. Importantly, *C. albicans* and *Strep. mutans* co-operate closely and share good synergy. This is very important as *Candida* is an extremely efficient user of carbohydrate energy, thus increasing the risk of caries development. Any treatment regime must therefore seek to balance these potential targets, which are not going to disappear.

Plaque is a choreographed continuation of simple to complex. Clinicians need to consider what stage the plaque has reached in an individual patient (Stages I to IV). If in Stage I or II, mechanical debridement will probably help, but the reality is that after moving from Stage II to III a variety of treatment options will be required. Stage IV is also a difficult stage but one that also has some options in management. We now know that plaque is not a single species, but is in fact composed of multiple species. The original Gram-positive group of bacteria form their link and are characterised by a cyclical nature of Stage I, II, III and IV. As we go from Gram-positives to more yeast and lactobacillus, these form the intermediary multi-species complex known as plaque, similarly characterised by four stages of maturity. The latter part of that is associated with the appearance of anaerobes, which are more often associated with deep periodontal pockets or subgingival infection. Thus, plaque is a choreographed multispecies environment that over time has as its consequence a different group of organisms. The management of plaque must therefore differ according to the stage of its presentation (e.g., Stage I vs Stage IV) and the patient's age group (e.g., 12 years vs 70 years of age). Only recently have we recognised the importance of this.

IV. Oral Hygiene Management

Using newer molecular methods to analyse the organisms found inside the matter blocking the airflow of the endotracheal tube, Prof Thomas' laboratory discovered that all were dental microbes. Moreover, the yeast (mycobiota) was extraordinarily yeast-specific and multiple varieties of yeast were found inside the endotracheal tube. All originated from the oral cavity. The importance of oral health care in the ICU becomes obvious when we are faced with the blockage of airflow in the endotracheal tube as a consequence of extensive amounts of oral flora. Who takes care of that obstruction? Whoever cleans the tube. Who cleans the mouth in the ICU? Nursing staff. Why not a dental hygienist? Who knows more about oral care of that patient?

This line of thought underlies the new concept of minimal intervention dentistry, which aims to put the patients in control so that they need minimal intervention from the profession.[6] This concept addresses the question of how to manage what we do without destroying the benefit of metagenomics.

Various available strategies can be used to control the biofilm via the habitats:

- Mechanical
  - remove/reduce/disrupt habitat
  - maintenance of oral hygiene
- Chemical
  - antibacterial
    - chlorhexidine, essential oils, triclosan, etc.
  - fluoride, xylitol, calcium, phosphate

Pre/Probiotic

Importantly, have you begun to consider organ transplant, i.e., a probiotic together with either mechanical or chemical strategies? We need to address the normal flora. In addition, it is essential to consider the different sizes of anti-biofilm molecules in oral health care. For instance, chlorhexidine molecules are large, associated with a molecular weight of 505.45 and a very limited penetration of the biofilm. Chlorhexidine is also a cationic agent, which means it is positively charged. In contrast, the essential oil eucalyptol has a much smaller molecular weight of 154.25, an almost neutral polarity and the capacity to penetrate the biofilm. Molecules that penetrate the biofilm will have all of these traits, remain stable over a variety of pH values, are generally neutral, are efficacious in a hydrophilic and hydrophobic environment, and generally require a carrier.

Thus, when performing an oral evaluation, no matter which age group of the patient, Prof Thomas and colleagues always ask these five questions:

- what is the size of the molecule being addressed
- is it stable at a huge pH range
- is it neutral to allow penetration to the biofilm
- is it workable in a dry and moist environment
- which molecule carries it?

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The first question that Prof Thomas asks is whether the particular product is good for the metagenomics or does it favour yeast. His institution continues to utilise essential oils, in the belief that these meet the criteria that best select for anti-biofilms. Molecules may not have the ability to reach the intended site of target.

How do we address the concept of tailoring comprehensive oral care from birth to death, in both inpatients and outpatients? Various methods are available:

1. To re-establish normal flora (metagenomics), the bioburden has to be reduced (but never to zero – the biofilm cannot be defeated).
2. Ventilator-associated pneumonia is a preventable oral-associated disease and we are letting it continue to be Number One in the US, costing $44,000 per patient because we do not take care of the mouth of an intubated patient.
3. Daily mechanical plaque removal.
4. Use of antiseptic mouth rinses. Notably, the mouth is ~25% abiotic surface (tooth) and ~75% sloughing biotic tissue. This has to have some means of reducing the bio-burden.
5. Professional mechanic debridement.
6. Soft tissues – the tongue is also an important reservoir of *C. albicans.* Choice of oral rinse therefore matters.

V. Changing Paradigms

Prof Thomas stressed that we are all in partnership with the bugs in our body. We are now beginning to realise that there are only four stages of our body in relationship to who we are by our bug population. When considered relative to dental risks and good health, we must consider the support concept that we all are different by age, i.e., therapy must be tailored by age of the patient, which addresses their microbial burden.

West Virginia instigated a very good programme with a large Federal grant that promoted oral health in little people, particularly in the 6–12-year-old group, called 'Happy Smiles'. In recognition of the fact that 80% of the US health budget is spent on infants aged <6 months and the >65-year-olds, Prof Thomas and colleagues initiated a programme to address the health of the aged patient in long-term care facilities.

The components of this programme involve:

- Aged care focus, recognising childhood oral care success
- Recognises return on investment and oral care
- Prior to ICU admission and in Unit
- Changing paradigm: dental hygiene utilisation

With this programme, dental hygiene becomes an integral part of oral care both within and outside the hospital in aged care.

In addition, a six-week programme with dental students who rotate in the ICU will be extended to include dental hygiene students from 2013. The programme aims to raise the focus of the health professional with the greatest expertise for the oral health of the patient. Prof Thomas' institution deals with dental care as an overall scheme. He believes there is a global need to regard oral health as a global index for a multitude of diseases ranging from birth to death.

Take home messages

We live in a microbial world: head to toe.

The distinction between dental and medical microbiology is a man-made fabrication via our simplistic attitude of a very complex total body ecosystem that is just now being uncovered.

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