REVIEW

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Biofilms and chronic rhinosinusitis: systematic review of evidence, current concepts and directions for research*

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SUMMARY

Introduction: *Chronic rhinosinusitis (CRS) possesses the hallmarks of biofilm mediated disease. This paper represents a systematic review of the published evidence for biofilms as the mediator of the inflammation in CRS. Current concepts on biofilm formation and properties, treatment strategies and directions for research are discussed.*

Methods: *A systematic review of the published literature for biofilms and their role in chronic rhinosinusitis was undertaken. Both Medline (1966-2006) and Embase (1988-2006) were searched until November 2006 which yielded 652 articles, 13 of which provided original research of biofilms in CRS.*

Results: *There were 7 studies demonstrating biofilm morphology in mucosal samples from human CRS patients. One study showed similar evidence for biofilms in an animal model of CRS. FISH techniques with CLSM were employed in one study to demonstrate biofilm formation in situ by S.pneumoniae, S.aureus, H.influenza and P.aeruginosa. In vitro biofilm forming capacity of microbiological samples, after culture, was assessed in two studies. Correlation with a clinical outcome was also made in these papers. One study demonstrated biofilm growth in removed frontal sinus stents.*

Conclusions: *Biofilms are associated with CRS, however, little research is available to define their role in the pathogenic process. There is tremendous potential for future research. Biofilms may be a significant factor in the inflammatory process.*

Keywords: biofilm, sinusitis, chronic, chronic rhinosinusitis, systematic review

INTRODUCTION

There is an evolving group of chronic infective conditions in which bacteria are forever present, difficult to culture and demonstrate consistently, resistant to current antimicrobial tools and often require surgical removal to resolve. This contrasts greatly with the acute bacterial infections that dominated medical practice until the mid twenty century [(1)]. To this burgeoning group of conditions, the antimicrobial tools that have evolved since the development of penicillin no longer represent a panacea for therapy. Despite adequate drainage, antibiotics are not the 'silver bullet' once envisaged for these chronic infective conditions [(2)].

The mucosa of the nasal airway and that of the paranasal sinuses is increasingly accepted as a single pathophysiological unit and thus the term 'rhinosinusitis' [(3)] is commonly used. A continuum also exists for pathology between the upper and lower respiratory tracts [(4)]. However, distinct clinical entities still exist with localised rhinosinusitis and more diffuse panrespiratory disease. The categorisation of chronic rhinosinusitis (CRS) by pathophysiologic mechanism is still largely undefined.

What mediates this prolonged inflammatory mucosal response? Even though allergy has always been implicated, evidence that atopy predisposes to chronic or acute rhinosinusitis is still lacking [(5,6)]. Other pathologic etiologies in chronic rhinosinusitis include asthma and leukotriene abnormalities, ciliary dysfunction, immune deficiency, ostial obstruction, bacteria, fungi, super-antigens (ie. exotoxins), osteitis and environmental factors [(5,7)]. Heterogeneity also exists between individual immune responses. The clinical spectrum of disease may partly be the result of individual CD4+ helper T-cell responses to antigens [(8)]. These are the Th1 and Th2 mediated immune responses [(8)].

A common pathogenic factor has been elusive and there is great variability in the pathophysiology that encompasses CRS.

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The concept of CRS, as a clinical entity, defining a chronic inflammatory endpoint, common to a range of pathogenic mechanisms is a popular concept [7]. However, recent evidence demonstrating biofilms of micro-organisms living within the paranasal sinuses has emerged as a theoretical common link in pathogenesis. These biofilms potentially provide a continual presentation of antigen (bacterial cell surface, fungal elements, exotoxin etc.) resulting in chronic inflammation.

'Free floating' or planktonic (see glossary in Table 1) describes a bacterium that acts independently, is readily mobile and often possess characteristics that allow for invasion of tissue. They are the phenotypic variants associated with acute infections. The sessile or biofilm bacterium works in collaboration, can be almost metabolically inactive and adopts a survival state. There has been a significant increase in research on biofilms over the past decade (Appendix A). Potentially more than 60% of infections currently treated involve biofilm formation [1]. Biofilm mediated infections are often chronic, are rarely resolved by host defences, are resistant to eradication even with directed antibiotics, are characterised by acute exacerbations and the microbial community is often difficult to define and culture. The clinical course of CRS often shares this profile. Can bacterial biofilms be used to explain the pathophysiology of CRS and the subsequent immune response dictating the clinical presentation?

Biofilm involvement has been described in otitis media, cholesteatoma and tonsillitis [9]. Otitis media has gained the most attention and the evidence for a direct biofilm mediated disease is mounting [10,11]. CRS possesses the hallmarks of biofilm mediated disease. But where is the evidence that biofilms are associated or even cause CRS? This paper represents a systematic review of the published evidence for biofilms as the mediator of the inflammation in CRS. Current concepts on biofilm formation and properties, treatment strategies and directions for research are discussed with relevance for rhinologic practice.

CURRENT CONCEPTS

Biofilm evolution

Biofilms are structured, specialised communities of adherent micro-organisms encased in a complex extra-cellular polymeric substance (EPS) [12]. The formation of biofilms is not restricted to bacteria, as fungal pathogens also form biofilms [13].

There is fossil evidence that micro-organisms have acquired the ability to form biofilms early in evolution. Morphology of biofilms have been identified in 3 billion year old sedimentary rock [14] and volcanogenic sulphur deposits [15].The ability of bacteria or fungi to form biofilms may represent an evolutionary adaptation. Protection from environmental factors (moisture changes, extremes of temperature, pH, UV light) in the harsh climate of earth, billions of years ago, would provide a survival advantage [16]. Avoidance of phagocytosis, the ability to concentrate nutrients [17], the development of close cell-to-cell signalling pathways and chemotactic motility [18] may have also contributed to biofilm formation. Bacteria live in biofilms

Table 1. Glossary of terms.

Biofilm	A biofilm is a complex aggregation of micro-organisms marked by the excretion of a protective and adhesive matrix. Biofilms are also often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interactions, and an extra-cellular matrix of polymeric substances
EPS	Extra cellular polymeric substances [9] Containing polysaccharides, proteins and nucleic acids Synonymous with: extracellular polysaccharide matrix [25] and exopolysaccharides
Polymeric (Greek ' many parts' polu-, *poly*- + meros, *part*)	Relating to Any of numerous natural and synthetic compounds of usually high molecular weight consisting of up to millions of repeated linked units, each a relatively light and simple molecule
Matrix	= EPS = the material or tissue in which more specialized structures are embedded
Planktonic phenotype	The name plankton is derived from the Greek word "planktos", meaning "wanderer" or "drifter" (Thurman 1997) or potentially like plankton, drifting organisms that inhabit the water of oceans, seas, and bodies of fresh water
Quorum sensing	The ability of bacteria to communicate and coordinate behaviour via signalling molecules. Similar to the legal definition, a quorum is the minimum number of members of a deliberative body necessary to conduct the business of that group.
Biofilm phenotype	Biofilm phenotype = sessile bacteria = biofilm type = non-planktonic form = a group of phenotypes that exist within a biofilm
Detachment/Dispersal	The loss of single cells or aggregates of cells from the biofilm. Detachment can be active (enzymatic or alterations in protein expression) or passive (mechanical force) process.
Th1 immune response (by CD4+ helper T cells)	Th1 responses produce interferon-γ, tumour necrosis factor α, interleukin(IL) 2, IL-12. This represents a cellular immunity against intracellular and viral pathogens [8].
Th2 immune response (by CD4+ helper T cells)	Th2 responses produce IL-4,5,6,9,10 and 13. The Th2 response mediates the humoral immunity against extra-cellular pathogens and antibody production. It is the Th2 mediated responses that dominate allergy and IgE production[8].
Knockout species	Genotype of a particular species that has been modified to remove or deactivate a particular gene expression. Thus being able to investigate the impact of the product the gene expression by its absence.

as a major and possibly preferred form. Biofilms represent greater than 90% of biomass in many environments [19].

Bacterial biofilms have a well recognised place in the etiology of periodonitis [20]. In 1683, Antoni van Leeuwenhoek's, well quoted, description of the 'tooth worm' or 'animalculi' from his own teeth represents the first description of a biofilm in disease [21]. Since then, biofilm associated infections with intravenous lines, orthopaedic implants and other implantable materials have been established [1]. Bacterial endocarditis, prostatitis and infectious kidney stones now have biofilms associated in their pathogenesis [22]. Mucosal based biofilm disease is an emerging field. There is greater difficultly with mucosal based disease to separate the complex microbial community that exists into pathogens and mere bystanders to the disease process. Demonstration of a 'biofilm specific' immune response mediating the inflammatory process may be the greatest challenge for mucosal based biofilm research.

Biofilm structure

The concept of bacteria locked in a slime, which protects against host defences and antibiotics, represents a gross misunderstanding of bacterial life in biofilm. While a physical understanding of the biofilm community is important, it is the organised functional heterogeneity [23], which enables the bacteria to take on a biofilm or sessile phenotype, that is the key.

There is no one common biofilm structure. The adaptation of bacteria into biofilms is both a response to environment and the genetic programming of the bacteria. Thus bacteria may form different biofilms on differing surfaces. The biofilm covering a mucosal surface will differ from the inert as it is in constant modulation by the host response [16]. Classically, the biofilm is described as sessile bacteria (15%)[24] enclosed in a well hydrated extracelluar polymeric substance (EPS) [25] or matrix (85%)[23]. The biofilm forms aggregates of EPS and bacteria into micro colonies. There are well developed channels [26] conveying fluid and nutrients by convective flow between these structures (Figure 1).

Polysaccharides (carbohydrate-rich polymers) and proteins constitute the majority of the EPS [28,29]. Nucleotides or extra-cellular DNA have also been demonstrated within the matrix [30]. Dead bacterial populations also form part of the structure [31].

Biofilm phenotype

It is the phenotypic change of bacteria living in biofilm that enables the organisms to adopt their most 'defensive' life strategy [18]. Environmental influences and cell-to-cell signalling are the two main factors which are likely to drive the bacteria into the biofilm phenotype. Local micro-environments within the matrix are thought to provide a stressed metabolic state [32]. Subsequent studies, using micro-catheters, have shown that oxygen and glucose gradients lead to metabolic depleted niches within the biofilm and subsequent phenotypic variations [33,34]. This results in groups of bacteria adapted to these environments. This variation in metabolic and functional phenotypes has been supported by analysis of bacterial protein production (proteomic study). Bacteria in the biofilm phenotype express many genes that planktonic forms never express [35]. Over 50% of the proteome can be up-regulated during biofilm formation. A diversity of phenotypes is also supported by these techniques [36]. Similarly, DNA micro-array assessment demonstrates distinct gene expression by biofilm bacteria compared to their planktonic counterparts [37-40].

Cell to cell signalling existing between bacteria in close proximity, called quorum sensing, facilitates the development of the biofilm phenotype [18]. Quorum sensing is an important factor in the initial formation of the biofilm along with the phenotype change and is discussed below. In fact, a single biofilm phenotype does not really exist. The biofilm phenotype refers to a collection of bacterial phenotypes that have been influenced by osmolarity, nutrient supply, cell signalling and population density. A vast diversity of bacterial characteristics may exist within the biofilm demonstrating phenotypic expression based on environmental factors [41].

Biofilm formation

The conversion of bacterial life, as free floating planktonic forms, to complex sessile communities has been extensively investigated. The process is one that has emerged from billions of years of evolution and likely to have multiple redundant pathways for its development. The local, low concentration signal production and reception in cell to cell signalling systems is called quorum sensing [42]. From proteomic studies of *Pseudomonas*, five main steps of development have been established [18,35] (See Figure 2).

The first stage involves attachment. During this process, surface contact sensoring is used to initiate a phenotypic change and the production of quorum sensing signals [43]. The second and third stage, adhesion and aggregation, involves the grouping and bonding of small numbers of bacteria [44]. This is facilitated by the presence of bacterial species specific characteristics such as Type IV pili of *Pseudomonas* [41]. The Type IV pili are required for a twitching activity of *Pseudomonas*. Mutant *Pseudomonas* species, without normal Type IV pili, lack the ability to aggregate [45,46]. This is particularly true within the high shear environment of a mucociliary blanket. Bacteria have great difficulty in accessing mucus covered tissues, especially when the mucous blanket is 200-250 μm thick and moves at speed [24]. Successful contact is followed promptly by EPS formation [47]. No absolute signals resulting in an 'on-off' effect have been identified for these steps. Signals for this process are likely to be multifactorial [16].

The fourth stage, Growth and Maturation, allows the redistribution of bacteria away from the substratum [42]. The complex architecture of the biofilm, including micro-colonies and water channels takes place. It is during this stage that various

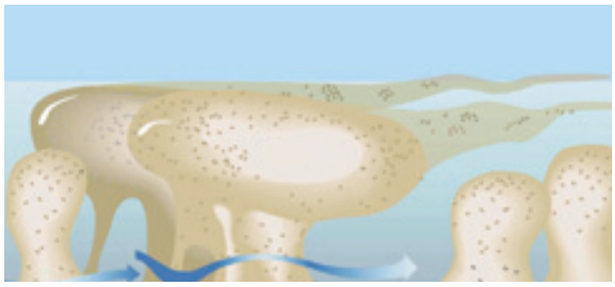

Figure 1. Basic biofilm structure demonstrating micro-colonies, water channels and detachment. Reproduced from [27] with permission, ASM Press.

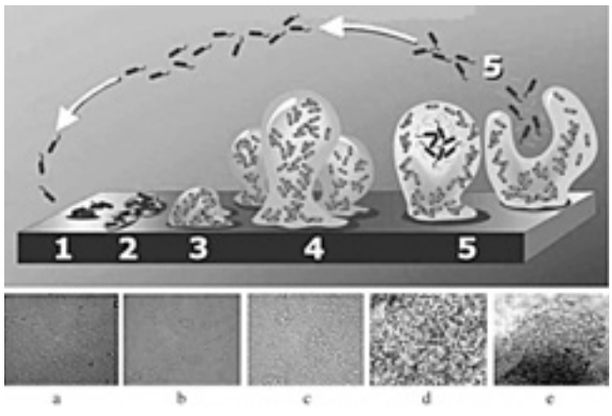

Figure 2. The biofilm life cycle: attachment, adhesion, aggregation, growth & maturation and detachment. Reprinted, with permission, from the Annual Review of Microbiology, Volume 56, ©2002 by Annual Reviews; www.annualreviews.org [18].

microenvironments and thus metabolic niches are established. This produces a diversity of phenotypes that live within the biofilm.

The final stage of detachment, or dispersal, can be an active or passive process. High shear environments can passively remove emboli from the biofilm colony itself or loosely aggregated planktonic organisms [18]. The former has the survival advantage of already being in the biofilm phenotype. The active process of detachment can involve enzymatic degradation of matrix or modulation of surface associate adhesions [48,49]. The by-products of EPS degradation may also facilitate other bacteria to be released as a planktonic form [17,50].

Biofilm formation represents a bacterial adaptation to the environment [16]. Both environment and bacterial genome contribute to biofilm development. The isolation of key gene sequences or products has proven elusive. Studies of knockout bacterial species, when certain biofilm specific genes have been 'switched off', still show persistent, although reduced, biofilm formation [30,51,52]. There is evidence of significant redundancy for these primitive pathways to biofilm formation [16] and a single on-off switch is unlikely [53].

BIOFILM AS A MEDIATOR OF MUCOSAL DISEASE

Clinically and pathologically, the processes of acute and chronic infection are distinct entities. However, many of the organisms are common to both. The ability of bacteria to choose which strategy they employ is a nascent field. *Pseudomonas aeruginosa*, for example, can cause acute auricular chondritis or otitis externa but can commonly be cultured from CRS and cystic fibrosis patients. Similarly, *Staphylococcal aureus* has the ability to facilitate abscess-forming folliculitis and sepsis yet is commonly seen in chronic osteomyelitis and CRS.

The role of planktonic and biofilm bacteria in initiating disease is significantly different. The invasion and motility properties of pseudomonal species in cystic fibrosis patients, is not expressed when in the biofilm phenotype [54]. Extra-cellular toxin production of *Pseudomonas*, a hallmark of acute pseudomonal infection and sepsis, is similarly not activated in the biofilm phenotype [55]. Whether acute and chronic infections are genetically distinct from the bacterial side or if they represent phenotypic variants only is unknown [56].

The host response to the biofilm, via surface antigens, exotoxins or EPS, is likely to differ greatly between individuals (Figure 3). The extent of collateral damage to host tissues, mediated by immune complex deposition and oxidative bursts from macrophages may be the major determinant in disease presentation [53]. Diseases where Th2 responses dominate, may benefit if a shift to Th1 responses could be made [57].

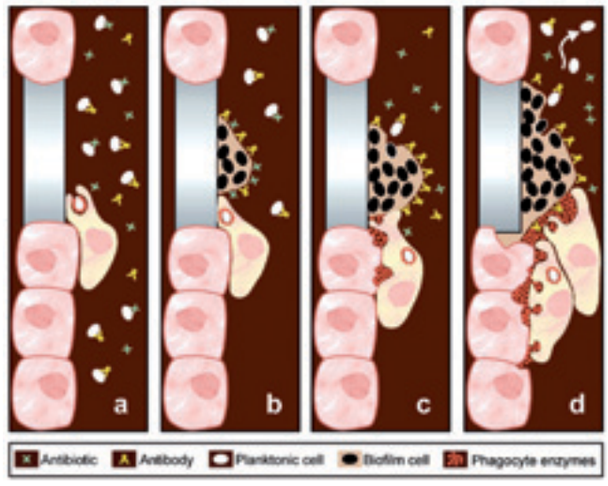

Figure 3. Diagram of a medical biofilm. **(A)** Planktonic bacteria can be cleared by antibodies and phagocytes, and are susceptible to antibiotics. **(B)** Adherent bacterial cells form biofilms preferentially on inert surfaces, and these sessile communities are resistant to antibodies, phagocytes, and antibiotics. **(C)** Phagocytes are attracted to the biofilms. Phagocytosis is frustrated but phagocytic enzymes are released. **(D)** Phagocytic enzymes damage tissue around the biofilm, and planktonic bacteria are released from the biofilm. Release may cause dissemination and acute infection in neighbouring tissue. Reprinted with permission from [1]. Copyright 1999 AAAS.

Antibiotic resistance in biofilm

Bacteria in biofilms have shown a remarkable resistance to chemically diverse biocides during defouling in industrial processes [58,59]. Difficultly in eradicating bacterial biofilm in medical conditions, such as infected implants, has proven their ability to avoid host and antibacterial efforts. It is speculated that this avoidance of host mechanisms was formed in the primitive earth against bacteriophages and free-living amoebae [18].

In biofilm disease, bacteria, when culturable, appear to be susceptible to common antimicrobials but these rarely eradicate the disease in the host. Early research focused on the surrounding EPS or matrix. Subsequently, many early concepts have been rejected and the biofilm phenotype is perceived as the main survival strategy [53].

The role of the EPS

Early research suggested that the EPS could retard diffusion of antibiotics [60] or slow diffusion due to osmotic gradients [61]. However, subsequent studies have demonstrated diffuse penetration of antibiotics [34,62] and most studies since have documented unimpaired antimicrobial penetration in the biofilm [62]. There are three exceptions: Beta-lactamase can accumulate in the matrix thus deactivating beta-lactam antibiotics [33], secondly, positively charged aminoglycosides are retarded by negatively charged matrix, like alginate from *Pseudomonas* [34], and thirdly, the EPS from coagulase negative *Staphylococci* reduces the efficacy of glycopeptide antibiotics [63].

The EPS was also once thought to obstruct neutrophils and antibodies from accessing the bacteria [64] and protect against radicals such as hydrogen peroxide [65]. Penetration by the host defence mechanisms into biofilms has subsequently been demonstrated. Antibodies and phagocytes can be visualised in the matrix [66]. Activated polymorphonuclear (PMN) cells are also seen within biofilm communities [67] (Figure 4). There may still be some role for deactivation of antimicrobials and reactive oxygen species, like those produced from phagocytic cells [68], but this appears to be a minor role in biofilm survival.

Phenotype studies have shown populations of bacteria in the EPS that are metabolically different to their planktonic counterparts. The effect of antibiotics can be seen on the surface of the biofilm but bacteria deep in the EPS are unaffected despite penetration of the antibiotic to these areas. Micro-catheter oxygen studies demonstrate that these areas within the biofilm are hypoxic and hypo-metabolic [34].

Quorum sensing contributes along with environmental factors. Biofilms that are too thin to allow a metabolically induced phenotypes still show antibiotic tolerance [69,70]. Genetically controlled phenotypes may be an important factor. This would allow the identification of genes which could prevent the biofilm phenotype and allow the persistence of a more 'antibiotic susceptible' planktonic form.

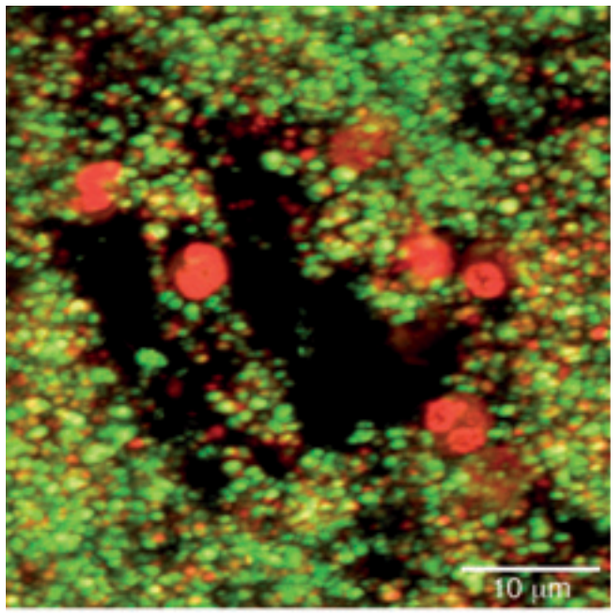

Figure 4. Confocal scanning-laser micrograph showing the invasion of a biofilm by polymorphonuclear cells (PMNs). The PMNs (large red nuclei) have entered the biofilm via the open water channels and have invaded short distances (1–5 µm) into the biofilm. The bacterial cells have been stained with the live/dead BacLight stain (BacLight Bacterial Viability kit; Molecular Probes, Eugene, Oregon, USA) and living bacterial cells (green) are seen in very close proximity (<1 µm) to PMNs. PMNs invade biofilms but are virtually inactive in killing sessile cells and resolving biofilm infections. Reproduced from [67] with permission, ASM Journals.

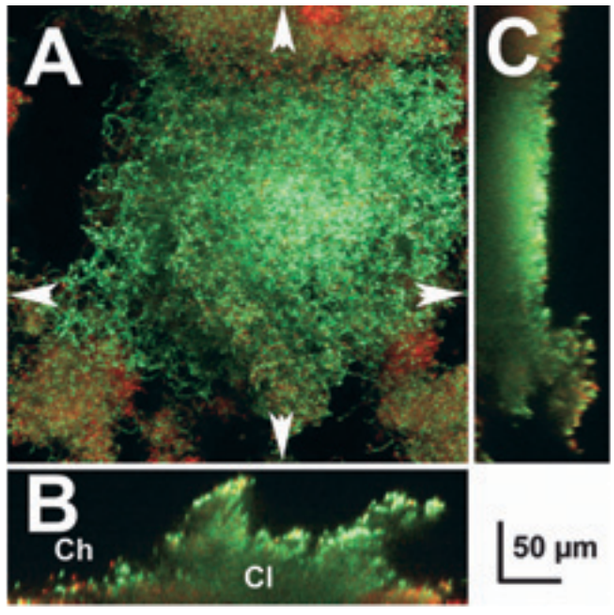

Figure 5. Biofilm formed from the dental plaque pathogen *Streptococcus mutans* showing characteristic cell clusters (Cl) made up of single cocci and chains separated by water. Reproduced from [9] with permission, LWW publishers.

Table 2. Identifying biofilm.
COMSTAT [73]*, Image Structure Analyzer (ISA)* [73].

Detection techniques for biofilms
Imaging:
• Scanning Electron Microscopy (SEM)
• Transmission Electron Microscopy (TEM)
• Confocal laser scanning microscopy (CLSM)
Imaging adjuncts (with CLSM)
• LIVE/DEAD Stain - BacLight Bacterial Viability Kit
• Fluorescent in situ hybridization (FISH) with species specific probes
Image processing (defines structure, mean thickness, roughness, substratum coverage and surface to volume ratio)
• COMSTAT image processing script for MATLAB [72]
• Image Structure Analyzer (ISA) [73]
Identification of non-planktonic bacteria
• PCR cloning
• Denaturing gradient gel electrophoresis (DGGE)
• Temperature gradient gel electrophoresis (TGGE)

Finally, the high numbers of bacteria in close proximity facilitates gene transfer. Conjugal or horizontal gene transfer is active in biofilms [71]. Plasmid transfer combined with long term and often recurrent antibiotic therapy, exposes the bacteria to strong selective pressures and allows the development of resistance [53].

Identification of biofilm

Standard imaging techniques can describe bacterial biofilm morphology well but offers little to describe the species present, gene expression or phenotype. Attempts to culture the bacteria, away from the environment from which they were sampled, will inevitably led to a change in phenotype. The use of species specific fluorescent in situ hybridization (FISH) enables the in situ identification of bacteria and their EPS. Molecular based bacterial detection techniques that utilise polymerase chain reaction such as PCR-cloning will allow non culturable bacteria to be defined [72]. Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) may also help to better define the microbial community within sinuses. Finally, computer aided analysis of confocal laser scanning microscopy (CLSM) enables conceptualisation of the complex 3-D biofilm structure [73] (Figure 5). A summary of identification techniques is present in Table 2.

Evidence for biofilms in CRS

Methods

A systematic review of the published literature for biofilms and their role in chronic rhinosinusitis was undertaken. Both Medline (1966-2006) and Embase (1988-2006) were search until November 21st 2006 which yielded 652 articles, 13 of which provided original research of biofilms in CRS. Details of the search and selection process are available in Appendix A.

Results

There were 7 studies demonstrating biofilm morphology in mucosal samples from human CRS patients [74-82]. One study showed similar evidence for biofilms in an animal model of CRS [81]. FISH techniques with CLSM was employed in one study to demonstrate biofilm formation in situ by *S.pneumoniae*, *S.aureus, H.influenza* and *P.aeruginosa* [82]. In vitro biofilm forming capacity of microbiological samples, after culture, was assessed in two studies [83,84]. Correlation with a clinical outcome was also made in these papers. One study demonstrated biofilm growth in removed frontal sinus stents [86]. A summary of the findings is included in Table 3.

DISCUSSION

It is likely that the microbial community in CRS is complex with the coexistence of biofilm and planktonic bacteria. No definitive physiological marker of the biofilm state for a bacterium exists and the detection of in-vivo biofilm growth by a particular species is difficult. Recent FISH studies on biopsies of sinus mucosa in CRS [82] and repeated in middle ear mucosa [11], represent species specific in situ identification of biofilms in disease states. However, these techniques still require mucosa ex vivo, are expensive, limited in assessing polymicrobial communities and require skill found at only a few centres. Parsek and Singh [22] suggested the following criteria to define infections caused by biofilms:

1. The infecting bacteria are adherent to some substratum or are surface associated.
2. Direct examination of infected tissue shows bacteria living in cell clusters, or microcolonies, encased in an extracellular matrix. The matrix may often be composed of bacterial and host components.
3. The infection is generally confined to a particular location. Although dissemination may occur, it is a secondary phenomenon.
4. The infection is difficult or impossible to eradicate with antibiotics despite the fact that the responsible organisms are susceptible to killing in the planktonic state.

Koch's postulate with a traditional 'swab and plate' census method for infectious agents no longer applies, not necessarily from a flaw in theory, but because our techniques to identify organisms in various phenotypic states has lagged behind our conceptualisation of the bacterial-host interaction. We propose that a fifth criterion be included, especially for polymicrobial communities existing in chronic mucosal disease:

5. There is a species specific host response that can be demonstrated from inflammatory changes or implied by a change of symptoms with corresponding alterations in the microbial community from treatment interventions.

There are limitations to these criteria; however, they provide general characteristics with which to consider the role of biofilms in human disease [22].

Table 3. Summary of published literature on the evidence of biofilms in CRS.

Study	Year	Population	Evidence for biofilms in CRS
Bendouah [83]	2006	Adults with CRS, n=19 Semi quantitative in vitro culture assessment	22 of 31 isolates of *S.Aureus, Coagulase negative Staphylococcal Species, P. aeruginosa* demonstrated biofilm forming capacity in vitro
Bendouah [84]	2006	Adults with CRS, n=19 Semi quantitative in vitro culture assessment	Correlates above data with a dichotomous outcome of 'poor or favourable' based on symptoms and endoscopic signs Poor outcome overrepresented in patients with biofilm forming isolates
Cryer [74]	2004	Adults with CRS, n=16 Biopsies of mucosa	Morphological evidence of EPS seen on four of the specimens and of bacteria in one on SEM
Ferguson [76]	2005	Adults with CRS, n=4 TEM of Biospies of mucosa	Morphologic evidence of biofilm in two of the samples on TEM Biofilm not seen in sinusitis model with bacteria defective with type IV pili (impaired attachment). Increased CBF seen in response.
Palmer [44]	2006	Rabbit model of P.aeruginosa, n=4, (type IV pili mutants) maxillary sinusitis SEM of mucosa	
Palmer [77]	2005	Review	Summary of the Perloff [81, 85] findings
Perloff [85]	2004	Frontal recess stents from post FESS adults n=6	All six had biofilm morphology on SEM and five had cultures with *S.aureus*
Perloff [81]	2005	Rabbit model of P.aeruginosa maxillary sinusitis, n=22	All clinically had sinusitis. 21 cultured *P.aeruginosa.* Morphological evidence of biofilm seen in all on SEM. No biofilm seen in the 22 contraleteral controls.
Ramadan [78]	2005	Adults with CRS, n=5 SEM of Mucosal biopsies	All 5 had morphological evidence of biofilms based on SEM.
Ramadan [86]	2006	Review	Summary includes data from Ramadan [78].
Sanclement [79]	2005	Adults with CRS, n=30, n=4 controls SEM/TEM of mucosal biopsies	Morphological evidence of biofilms seen in 24 patients. No controls positive.
Sanderson [82]	2006	Adults with CRS, n = 18, n=5 controls FISH visualised with confocal microscopy of mucosal biopsies	FISH for *S.pneumoniae, S.aureus, H.influenza* and *P.aeruginosa* and standard cultures. 14 of 18 had evidence of biofilm and 2 of 5 controls. Cultures did not correlate.
Zuliani [80]	2006	Children with CRS and OSA, n =16 Adenoid samples	All 8 CRS patients had biofilms in the adenoids. No control demonstrated significant biofilm coverage.

CRS biofilm research has demonstrated that biofilms do exist on the mucosa of patients. Electronic microscopic study has demonstrated morphological evidence of biofilms in both human CRS patients and animals models of CRS. This has been confirmed with species specific FISH studies. The role that these biofilms play in the inflammatory cycle is still unknown. Bendouah [84] represents the only study to link biofilm factors to clinical outcomes. However, bacterial samples were cultured and re-cultured in vitro to test for biofilm forming properties. It is unlikely that the phenotype expressed in vitro was similar to that in vivo. It is also acknowledged that biofilms on inert and mucosal surfaces will be morphologically and functionally different [16]. Because of phenotypic variation, the results of this study are difficult to interpret.

FUTURE THERAPY

How will otolaryngologists take this knowledge and convert it to treatment strategies? Novel and traditional therapies may both have a role in targeting biofilm related disease. Due to the survival advantage of bacteria in biofilm phenotype, the prospect of locking bacteria into a planktonic form and thus improving antiobiotic susceptibility is one possibility. This may be achieved by interventions at various stages of biofilm development.

Targeting the surface

There is little research on the molecular assessment of mucosal surfaces in sinuses. A better understanding of the microbial community and biofilms that exist in normal mucosa is required. FISH analysis of normal mucosa had shown *H.influenza* biofilm in 2 of 5 samples [82]. Similar findings have also been found in the control samples of normal contra-lateral mucosa of animals with otitis media [87]. However, the presence of biofilm in healthy sinuses is not supported by imaging. Sanclement and Perloff [79,81] could not find morphological evidence of biofilm in their controls. There are significant gaps in our knowledge about the natural ecosystem of the paranasal sinuses. The role of natural biofilm systems in the nose and sinuses and their prevention of pathogenic biofilm formation requires further research [9].

Altering the matrix

The visco-elastic or hydrogel properties of the biofilm, which protects against shear and mechanical stress, have not been widely investigated [88]. The current use of saline irrigation, sprays and douching may have a role in the mechanical removal of biofilm. The matrix may be further manipulated with additives to irrigation fluid to improve efficacy. Changes in mucus rheology and surfactant [89,90] may also prevent biofilm formation.

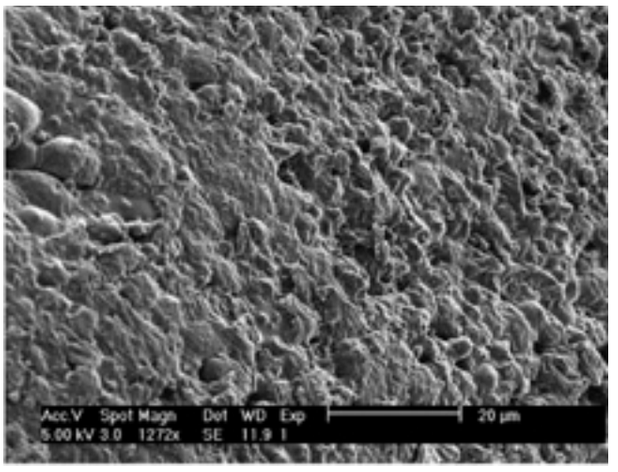

Figure 6. Biposy specimen of human maxillary sinus mucosa demonstrating the morphology of Pseudomonas mucosal biofilm. Reprinted from [77]. Copyright 2005, with permission from Elsevier.

Potential for altering the EPS may also exist. Polysaccharide intercellular adhesion (PIA) is a major matrix component in staphylococcal biofilm. Knockout staphylococcal biofilms, lacking PIA, are more susceptible to PMN phagocytosis and death [91]. Changes in the matrix may bring about phenotypic changes if metabolic niches are removed.

Improving susceptibility to antimicrobials

The efficacy of antibiotics could be improved if changes to the metabolic states of the biofilm bacteria could be made. Simple attempts to deliver high concentrations or prolonged courses of antimicrobials are unlikely to be effective. This approach is likely to result in increased resistance patterns as discussed previously. Treatment of biofilm disease follows a path unfamiliar to traditional infectious disease thinking. Novel therapies have shown improved antibiotic susceptibility of biofilm bacteria in both electric fields [92,93] and from ultrasonic stimulation [94,95].

Arresting the steps to biofilm formation

The prevention of biofilm formation perhaps has the greatest promise as a treatment goal. Unfortunately, an 'on-off' switch is unlikely to be found. The transition of bacteria to biofilm is a process embedded in billions of years of evolution and is likely to have many redundant pathways [16]. Some compounds have been shown to reduce biofilm formation [96,97]. Direct inhibition to quorum signalling, by antagonists, have been the obvious target [98,99].

Acylated homoserine lactones (AHLs) are the predominate quorum sensing signal in gram negative bacteria [100]. In Gram-positive bacteria, species-specific quorum sensing is mostly facilitated through small peptides [101]. Recently, interspecies communication has been linked to autoinducer-2 (AI-2) [102]. In Gram-positive biofilm Ribonucleic-acid-III-inhibiting peptide (RIP) has shown the ability to block biofilm formation by *S.aureus* and *S.epidermidis* [103-105]. Quorum sensing antagonists might lock a significant proportion of the bacteria into a planktonic mode and allow antibiotic susceptibility. This process is being tested with RIP and antibiotic impregnated vascular grafts [103].

Plants have recognised symbiotic relationships with colonising bacteria. They also use quorum sensing signalling to control this process. There may be natural plant based quorum sensing signals to control pathogens [106].

Thus far we have been able to block quorum sensing and make use of isolated components for driving protein expression. However, the full-scale manipulation of the bacterial quorum circuit in a biotechnological application remains an unfulfilled goal [101].

THE NEXT STAGE

The need to identify non-planktonic bacteria in health and disease, defining the role of natural biofilm systems and demonstrating a biofilm specific host response in CRS remain to be established. Substances that prevent adhesion, inhibit quorum signalling and modulate the matrix are likely to make good treatment adjuncts. In the interim, it may rest on our ability to manipulate the host response, Th1 and Th2 immune responses to the antigenic stimulation by the biofilm, until our understanding of biofilm life matures.

A list of web resources is listed in Appendix A, which is available on our website: www.rhinologyjournal.com

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