is not always linked to the presence of proteins containing the sortase substrate, an LPXTG motif[4].

These observations illustrate the fact that bacteria have developed different cell-surface-display strategies throughout evolution, and that a universal wall-anchoring mechanism is unlikely to exist. Accumulating data indeed show that distinct wall-anchoring mechanisms can exist in a given bacterium, either simultaneously (e.g. the InlA and InlB proteins from *Listeria monocytogenes*) or sequentially, following changes in cell wall composition (e.g. the SbsA and SbsB proteins from *B. stearothermophilus*).

Finally, another issue raised by the analysis of wall-anchoring-deficient mutants in *B. anthracis* concerns the crucial role of wall-associated polymers in bacterial physiology. Within the context of the development of new antimicrobial strategies, wall-associated polymers should undoubtedly be considered as potential targets. Therefore, we suggest that the terms 'secondary' or 'accessory' polymers should be avoided and replaced by 'peptidoglycan-' or 'wall-associated' polymers.

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Cystic fibrosis pathogenesis and the role of biofilms in persistent infection

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Recent work has presented definitive evidence that the *Pseudomonas* cells that infect the lung in cystic fibrosis grow in the biofilm phenotype. These unequivocal data establish this chronic infection of compromised hosts as the archetypal biofilm infection, which is both refractory to antibiotic therapy and barely affected by host defenses.

The direct examination of a very wide variety of natural and industrial environments by the NSF-funded (ECD-8907039) Center for Biofilm Engineering has shown that the majority of bacteria in these ecosystems grow in matrix-enclosed communities and adopt a distinct biofilm phenotype. Because these prokaryotic cells cannot be aware of their location, vis-à-vis human beings, microbial ecologists have long speculated that this inherently defensive mode of growth must also predominate in the hospital environment and in the tissues of infected patients. As the list of distinct characteristics of the biofilm phenotype lengthens, and as direct morphological techniques improve, we can characterize more infections as being caused by biofilms and can bring anti-biofilm strategies that have been developed in industry to bear in the medical sector.

A recent paper by Singh *et al.*[1] adds two important increments of data to support the burgeoning hypothesis that the chronic pneumonia that affects cystic fibrosis (CF) patients is caused by *Pseudomonas aeruginosa* growing in biofilms. The first data are morphological, and show that *P. aeruginosa* cells recovered directly from sputum of CF patients are arranged in micro-colonies, in which all of the cells are enveloped within a thick matrix material. In this paper, the matrix material was protected from the dehydration necessary for examination by electron microscopy by fixation with perfluorocarbon before normal fixation and dehydration, and the resultant images show the protective matrix with great clarity. These images supersede earlier electron microscopic images[2] in which the authors 'reconstructed' the concept of thick matrices from the radically condensed remnants of preparations that had been stained with ruthenium red following conventional dehydration. They also support the light-microscopic images of *Pseudomonas* cells in CF sputum that show Gram-negative cells within large areas of homogenous material interpreted (correctly as it transpires) as matrix material by Hoiby's group[3].

The second increment of data in the Singh *et al.* paper uses the new criterion of the ratio of the two quorum signals produced by *P. aeruginosa* cells to support the hypothesis that these pathogens grow as biofilms in the CF lung. Greenberg's group[4] showed that *P. aeruginosa* cells produce quorum signals that are controlled by the *las* and *rhl* genes, and Davies *et al.*[5] showed that one of these systems (Las) controls biofilm formation by cells of this species. These published data inferred that the relative levels of these quorum signals would be different in planktonic and biofilm populations of *P. aeruginosa*, but definitive proof had not yet been presented. Singh *et al.* present *in vitro* data showing that biofilm cells produce more of the butyryl (C4) acyl homoserine lactone (AHL) than the oxyodecanoyl (C12) signal, whereas planktonic cells produce the same two signals in the reverse ratio, favoring the C12 signal. Direct examination of sputum from CF patients showed the biofilm pattern of signal production, in that more of the C4 signal was present, in approximately the same ratio as that seen in biofilms grown *in vitro*. These data support the hypothesis that *P. aeruginosa* grows in biofilms in the CF lung, and they are especially valuable because they use quantitative chemical methods where previous support for the biofilm hypothesis was largely morphological.

Significance of the biofilm hypothesis in chronic bacterial infections

Based on direct examinations of materials from device-related and other chronic

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Table 1. Partial list of human infections involving biofilms[a]

Infection or disease	Common biofilm bacterial species
Nosocomial infections:	
ICU pneumonia	Gram-negative rods
Sutures	*Staphylococcus epidermidis* and *Staphylococcus aureus*
Exit sites	*S. epidermidis* and *S. aureus*
Arteriovenous shunts	*S. epidermidis* and *S. aureus*
Schleral buckles	Gram-positive cocci
Contact lens	*Pseudomonas aeruginosa* and Gram-positive cocci
Urinary catheter cystitis	*Escherichia coli* and other Gram-negative rods
Peritoneal dialysis (CAPD) peritonitis	A variety of bacteria and fungi
IUDs	*Actinomyces israelii* and many others
Endotracheal tubes	A variety of bacteria and fungi
Hickman catheters	*S. epidermidis* and *Candida albicans*
Central venous catheters	*S. epidermidis* and others
Mechanical heart valves	*S. aureus* and *S. epidermidis*
Vascular grafts	Gram-positive cocci
Biliary stent blockage	A variety of enteric bacteria and fungi
Orthopedic devices	*S. aureus* and *S. epidermidis*
Penile prostheses	*S. aureus* and *S. epidermidis*
Dental caries	Acidogenic Gram-positive cocci (e.g. *Streptococcus*)
Periodontitis	Gram-negative anaerobic oral bacteria
Otitis media	Non-typable strains of *Haemophilus influenzae*
Musculoskeletal infections	Gram-positive cocci (e.g. staphylococci)
Necrotizing fasciitis	Group A streptococci
Biliary tract infection	Enteric bacteria (e.g. *E. coli*)
Osteomyelitis	Various bacteria and fungal species – often mixed
Bacterial prostatitis	*E. coli* and other Gram-negative bacteria
Native valve endocarditis	Viridans group streptococci
Cystic fibrosis pneumonia	*P. aeruginosa* and *Burkholderia cepacia*

[a]Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; ICU, intensive care unit; IUD, intrauterine device.

infections, and on patterns of inherent resistance to antibiotics and to host clearance mechanisms, the US Center for Disease Control (CDC) has estimated that biofilms cause 65% of infections in the developed world (Table 1). This general hypothesis of the biofilm etiology of chronic bacterial diseases, articulated most recently by Costerton *et al.*[6], is largely based on morphological data showing that the infecting organisms grow in matrix-enclosed micro-colonies, similar to those seen in the Singh paper. The addition of this new and direct chemical data, in addition to very strong morphological data, now makes CF the chronic bacterial disease that will be used as the 'gold standard' against which each putative biofilm disease will be assessed for inclusion in this burgeoning classification. We have speculated that the emergence of biofilm diseases, to comprise a majority of infections in the developed world, is the result of the success of vaccines and antibiotics designed against planktonic bacteria in controlling acute diseases caused by these free-floating cells[6].

The considerable practical importance of the impetus provided by the work of Singh *et al.* is the provision of an intellectual nucleus around which the concept of biofilm infections can now be built. Until this time, each putative biofilm infection has been included in the list based on morphological data, and sometimes on its clinical recalcitrance, but the addition of unequivocal chemical data has now established CF pneumonia as the definitive biofilm infection. As research in this area proceeds, many more chronic infections (e.g. otitis media) will be defined as being biofilm infections, based on the direct chemical analysis of signal molecules or of genotypic analyses showing the expression of biofilm-specific genes. Chronic bacterial infections on the biofilm infection list can then be cross-referenced, because of etiological similarities, to transfer useful concepts from one biofilm disease to another.

One example would be to examine immunological data from patients with infectious prostatitis, in the light of data showing that CF patients suffer severe tissue damage from the production of immune complexes[7], and resultant 'frustrated phagocytosis'[6]. Immune suppression has been helpful in some CF patients, and it might have similar usefulness in prostatitis, or even in other chronic infections such as osteomyelitis. Another example would be the administration of very high doses of antibiotics, which has been helpful in the treatment of native valve endocarditis and of infections of hip prostheses, by direct infusion through tubes in the tympanic membrane in otitis media with effusion (OME). It is axiomatic that we can begin to exchange therapeutic strategies between recognized biofilm infections, when the etiologies and clinical properties of the chronic diseases on the list (Table 1) have been shown to be similar or identical.

Useful importation of biofilm concepts from adjacent fields

The adoption of biofilm concepts in medical microbiology has lagged far behind the acceptance of these new notions of bacterial growth in adjacent areas of research, such as microbial ecology and industrial microbiology. As the biofilm concept is transferred to medicine, with the notion that bacteria follow a similar growth strategy in all ecosystems, a large body of very exciting research can be transferred into the medical area. Hundreds of modern papers describing the structure and function of biofilms formed by cells of *P. aeruginosa*[8] can be added to the medical lexicon, as soon as we conclude that the cells of this organism that grow in infected tissues have truly adopted the biofilm phentoype. Along with the biofilm concept, medical microbiology will be able to assimilate new, direct methods of studying biofilms from the parallel biofilm fields in science and engineering, once the biofilm phenotype has been established in the bacteria that actually cause chronic infections. These methods are based on confocal microscopy and they include the species identification and location of the bacteria present, the physiological activity of each cell, the viability of each cell, the local production of signal molecules and the expression of specific genes as indicated by reporter constructs. Biofilm engineers have developed physical probes to measure local pH and oxygen concentrations[9], and a new confocal probe that allows us to visualize the structure and activity of biofilms *in situ* in infected organs.

Concluding remarks

Perhaps the most exciting consequence of the developing acceptance of the biofilm phenotype in chronic infections is the immediate possibility of medical benefit from the three decades of experience that ecological and industrial biofilm researchers have in biofilm control. Biofilm researchers have learned that continuous small doses of conventional antibacterial agents (biocides and antibiotics) are ineffective and likely to induce resistance, so they have turned increasingly to bolus doses delivered directly to the biofilm to be controlled. They depend on direct observations of biofilm populations to detect complete killing, because they know that regrowth rates of partially killed biofilms are very high. Biofilm researchers have discovered that low-intensity DC electric fields[10] and certain ultrasonic frequencies (W.G. Pitt, pers. commun.), can reduce the antibiotic resistance of biofilm bacteria to that of planktonic cells, and technologies based on these discoveries are in commercial development. Biofilm researchers have also discovered that the behavior of biofilms is controlled by chemical signals (which tend to be AHLs in Gram-negative organisms), and they have begun to manipulate both biofilm formation and detachment using these signals and their analogs. Already, we have discovered signals that control biofilm formation in the marine environment[11], signals that induce the detachment of planktonic cells from mature biofilms (P. Stoodley, pers. commun.), and signal analogs that block toxin production by specific biofilm bacteria. The societal impact of biofilm research, if the medical team can be linked to the existing biofilm community of scientists and engineers, begins to beggar the imagination. The Singh *et al.* work published recently in *Nature* brings that happy day much closer.

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Leprosy lipid provides the key to Schwann cell entry

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A recent study has demonstrated that the species-specific phenolic glycolipid of *Mycobacterium leprae* triggers uptake into Schwann cells by interaction with laminin-2 and the α-dystroglycan receptor. This finding emphasizes the importance of lipids in the biology of mycobacterial infection and suggests possible strategies to combat nerve damage in leprosy.

'*Let me have about me men that are fat...*' *Julius Caesar* Act 1, scene 2.

Had Julius Caesar avoided the sharp end of republican politics, he would surely have succumbed to infection with mycobacteria – captains of all the men of fat. During the dark night of the mycobacterial research soul that followed the assumed conquest of tuberculosis (TB) in the 1950s, biochemical analysis of the unique mycobacterial cell wall lipids and carbohydrates provided the mainstay for continued scientific interest in these organisms. By contrast, the research revival from the mid-1980s – first with leprosy, and then TB – focused on DNA and proteins. Rapidly developing molecular genetic techniques were accurately perceived as offering new opportunities for analysis of the slow-growing pathogens that had proved such difficult targets for conventional microbiology; peptides were seen as the only valid currency in the realm of the T-cell-mediated responses required for mycobacterial immunity. Inexorably, however, lipids have been floating back to the top of the mycobacterial research agenda.

Lipids and mycobacterial pathogenesis

The genome sequence of *Mycobacterium tuberculosis* revealed an abundance of genes encoding proteins involved in the synthesis and degradation of lipids, with representation of metabolic strategies previously found in a range of microorganisms, mammals and plants, in addition to pathways unique to the mycobacteria[1]. Elucidation of the mechanisms of antimycobacterial drug action contributed to the characterization of pathways for the production and assembly of mycolic acids (long-chain branched fatty acids characteristic of the mycobacterial cell wall), and the corresponding biosynthetic enzymes are promising targets for development of new agents to combat the emergence of multidrug-resistant isolates[2]. The application of genetic tools, such as signature-tagged mutagenesis, to probe the mechanisms of mycobacterial virulence has highlighted the importance of lipids during the process of infection. Mutations that disrupt biosynthesis of a wax ester present on the outer surface of *M. tuberculosis* (phthiocerol dimycocerosate, PDIM)[3,4], or that alter the precise structure of mycolic acids[5], are associated with a reduction in disease in animal models, and lipids play a crucial role as nutrients during the prolonged course of infection[6]. In terms of

  PII: S0966-842X(00)01917-X