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Principles of antimicrobial biomaterials: why they do not (usually) work

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A very extensive range of implantable devices is now available, each with different risks for infection. In terms of prevention of infection, we need to pay particular attention to its instigation, and in order to target prevention strategies accurately we need to know the period of risk to the device. This can be clarified by categorizing devices according to whether they are completely implanted and long-term (e.g. hip replacements), partially implanted (e.g. central venous catheters) or not implanted (e.g. urinary catheters). The period of risk is then seen to vary from the time of surgical insertion only, to throughout the life of the device. Prophylactic antibiotics are useful only in those devices where the period of risk is mainly at insertion. Antimicrobial biomaterials are therefore worthy of consideration.

Types of antimicrobial biomaterials include surface-treated, coated, admixed and impregnated; the agents include antibiotics and metals such as silver. The behaviour of the agents in impregnated biomaterials is crucial to their preventative activity. This can be predicted by such factors as the melting point of the agent and its solubility in a solvent in which the uncross-linked polymer is soluble (Hansen index). Equally important is a consideration of the events in instigation of implant infection, such as conditioning film, as they affect the function of the antimicrobials. An important principle of antimicrobial biomaterials is that the activity must be retained at the interface with the tissue, and should not be intended to give rise to measurable tissue concentrations. This avoids systemic toxicity and other side-effects. The layer of antimicrobial molecules or metal ions at this interface is the Nernst layer, which must be sustained in the face of fluid flow and protein conditioning film in order to exert clinically useful antimicrobial activity for the necessary period. Only impregnated biomaterials achieve this. Silver coatings have an additional problem in that the silver ions are rapidly converted to silver chloride and this has a very low dissociation constant. Silver toxicity may result if metal particles or salts are allowed to leach into the surrounding tissues.

The type of antimicrobial biomaterial must be designed to address the in-use period of risk and a decision must be taken on whether this is always possible or desirable.

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Characterization of bacterial adhesion onto surfaces of stainless steel by micromanipulation

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Bacterial adhesion to surfaces is a widespread phenomenon. For many years protocols used to evaluate the effectiveness of disinfectants have been carried out on bacteria in suspension. However, the efficiency of some disinfectants is reduced by up to 1000-fold when bacteria are present in a biofilm.

Bacterial accumulation into a biofilm is the net result of a number of physical, chemical and biological processes. These processes are related to the level of adhesion between bacteria and substrate. Adhesive strength between bacteria and substrate can be defined as the work required to remove the organisms per unit area from the surface to which they are attached. Understanding the adhesive strengths between bacteria and surfaces is fundamental in terms of improving strategies for their removal.

A micromanipulation technique has been developed which characterizes the adhesive strengths of biofilms and biomass to substrates. Advantages of this technique include the direct measurement of the force required to remove biofilms and biomass off substrates, allowing investigation of the relationship between environmental factors and bacterial adhesion. In addition the cohesive nature of the bacterial communities can be compared to determine mechanisms of attachment. Such information is very relevant to the development of cleaning strategies and in elucidating modes of action.

Biofilms of *Pseudomonas fluorescens* grown on stainless steel substrates were prepared using a minimal media. Levels of adhesion between biofilm and substrates were determined using the micromanipulation technique. Adhesion measurements were carried out to determine the effects of growth stage, glucose concentration, pH, and substrate roughness. Results showed adhesion was greatest at the stationary phase of biofilm growth at a glucose concentration of 6 g/l maintained at pH 7. It was also found that adhesion increased with an increase of the surface roughness. Micromanipulation was used to determine the effectiveness of 18 separate detergent preparations. Results showed reproducible evidence of which detergents acted the most effectively on a series of different substrates.

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Yersinia spp./*C. elegans* infection model: biofilms on a biotic surface

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The outcome of infection by pathogenic bacteria depends on virulence factors expressed by the bacteria, the existence of corresponding host targets and host responses to these factors. The usual models for studying these interactions (mouse, mammalian cell culture) are genetically unwieldy and do not lend themselves to experimental analysis. Also, there are cost and ethical restraints on the use of laboratory mammals. The idea of using a non-mammalian and genetically tractable host organism is therefore attractive.

To investigate the pathogenicity of *Yersinia* spp. and the evolutionary divergence of the genus, we studied the effect of pathogenic yersiniae on the model organism *Caenorhabditis elegans*, a worm. We found that both *Yersinia pestis* and *Yersinia pseudotuberculosis*, but not *Yersinia enterocolitica* cause

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blockage of the *C. elegans* gut and eventual death of the worm. Electron microscopy and cytochemical examination of infected worms indicated that the infection phenotype was the result of biofilm formation over the head of the worm. Seven transposon mutants of *Y. pseudotuberculosis* strain YPIII pIB1 were completely or partially attenuated; mutated genes included genes coding for haemin storage and lipopolysaccharide biosynthesis. A screen of 15 defined *C. elegans* mutants identified four where mutation caused (complete) resistance to infection by *Y. pseudotuberculosis* YPIII pIB1. These mutants, *srf-2*, *srf-3*, *srf-5* and the dauer pathway gene *daf-1*, also exhibited altered binding of lectins to the nematode surface.

Mutations of specific bacterial and nematode genes indicate that biofilm formation is under genetic control and suggest that biofilm formation on a biotic surface is an interactive process involving both bacterial and invertebrate control mechanisms.

Periodontal pathogens in dental unit waterlines

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Beside the well-known biofilm contamination by water organisms colonizing waterlines within dental units, another source of contamination has been shown where microorganisms are retracted from the patient's oral cavity through a "suck-back" that occurs during high-speed hand-piece use. To minimize the potential health hazard deriving from patient-to-patient cross-contamination, the American Dental Association (ADA) has repeatedly urged manufacturers to install anti-retraction valves in new dental units, and encouraged oral practitioners to flush waterlines between patients in order to eliminate any microorganisms eventually retracted during a previous visit. The aim of these studies was to evaluate the efficacy of ADA recommendations in controlling patient-to-patient contamination through dental unit waterlines, and to search for the presence of human pathogens in dental practice units.

Anti-retraction valves provided initial important benefits, but, within a few weeks of use, 90% of them showed a dramatic failure in opposing fluid retraction, giving rise to some concern about how long these devices will function reliably after installation. As regards the between-patient mechanical flushing, the results of the same study showed that the procedure is only effective in reducing by one $\log_{10}$ the microorganisms present in waterlines before flushing. This may assume particular clinical relevance, since it could be possible to retract as much as 5–6 $\log_{10}$ of oral pathogens into waterlines during dental operations and, in that case, there would still be 4–5 $\log_{10}$ remaining within waterlines after as much as 2 minutes of flushing. In the second part of the study, we evaluated the presence of DNA from *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* and *Treponema denticola*. The results showed that two specimens tested positive for *P. intermedia* and both were obtained from dental units used with high-speed dental hand-pieces used directly inside the mouth.

Dental units have the potential to retract and transmit human pathogens and recommendations fail to control patient-to-patient cross-infection. Manufacturers should be invited to design dental units that incorporate automated devices to disinfect waterlines between patients with minimal effort for the dental staff.

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Biofilms as reservoirs of antibiotic resistance

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The aim of this work was to answer three specific questions regarding the potential of oral bacterial biofilms to act as reservoirs of antibiotic resistance genes. (1) Is antibiotic resistance prevalent in the oral microflora? (2) What are the genetic supports for antibiotic resistance genes in the oral flora? (3) Do any of the resistance genes transfer within oral biofilms?

Antibiotic resistance was found to be common in samples of plaque taken from healthy adult volunteers, all individuals harbored antibiotic resistant bacteria. Gentamycin resistance was the most common (23% of total cultivable flora), followed by erythromycin (18% of total cultivable flora) and tetracycline (10% of total cultivable flora). The *tet*(M) gene was found to be responsible for most of the tetracycline resistance followed by *tet*(W) *tet*(O) *tet*(Q) then *tet*(S). These genes were found in most of the major oral species. The most common gene responsible for erythromycin resistance was *mef* followed by *erm*(B) and *erm*(F). Again these genes were found in the major oral species. The genetic supports for some of these resistance genes was investigated. It was found that *tet*(M) was usually located on a Tn*916*-like conjugative transposon, the *erm*(B) gene was also often found on such a transposon and it was common to find *erm*(B) and *tet*(M) on the same conjugative transposon. Some of these conjugative transposons were shown to be able to transfer to other recipients in model oral biofilms. One of the *tet*(W) genes was shown to be contained within a novel transposon structure. However, we could not transfer this gene in model oral biofilms or on filters.

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Systemic antimicrobials and periodontitis – a classic biofilm disease

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Periodontitis is a group of infectious diseases characterized by a hyper-inflammatory response to the dental plaque biofilm. In view of the primary bacterial aetiology, there has been great interest in employing systemic antimicrobials, since mechanical treatment alone is not always successful. There is a diversity of research findings addressing this problem and a lack of clarity regarding possible adverse effects from antimicrobials.

We developed rigorous systematic review methods to test the null hypothesis: "There is no difference between adjunctive treatment of periodontitis with and without antimicrobials in terms of clinical, patient centred and adverse outcomes". A detailed search included multiple electronic databases, bibliographies of found papers and review articles. Studies were randomized or controlled clinical trials. Where studies were similar enough, meta-analysis was conducted and causes of heterogeneity investigated.

A total of 1300 records were examined and 22 clinical trials were relevant to the review. Seven antimicrobials or combinations were found. Only spiramycin (probing depth change 0.41 mm 95% CI 0.08, 0.73) or the combination of metronidazole and amoxicillin (probing depth change 0.45 mm 95% CI 0.19, 0.71) produced a statistically significant benefit in favour of the drug group, although the magnitude of the difference was small and the clinical relevance

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unclear. Similar results were found if all antimicrobials were included in one analysis. Within the clinical trials, only one study examined the change in bacterial resistance. This demonstrated a transient increase that had disappeared after 6 months.

Currently, there is little place for systemic antimicrobials in periodontal therapy. For each antimicrobial, there are few randomized placebo-controlled trials. Of the existing data, some show a small improvement over standard treatment. There remains, however, no clarity on choice of drug, dosage, clinical relevance of the benefits or safety. Future research should employ rigorous methods to evaluate antimicrobials in high risk or poorly responsive patients. In addition, future studies should investigate markers (microbiological, genetic) that might predict responders. Safety of antimicrobials must be carefully investigated.

Prion disease in the dental setting

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The prion diseases are a group of rare fatal neurodegenerative disorders of humans and animals, histopathologically characterized by spongiform change of the central nervous system. Sporadic Creutzfeldt–Jakob disease (CJD) is the most common of the acquired human prion disorders, giving rise to rapid onset dementia in elderly persons worldwide. Sporadic CJD may arise as a consequence of spontaneous mutation within the prion protein coding gene (*PRNP*) on chromosome 20. In contrast, the recently described variant CJD (vCJD) has affected young adults from Western Europe, giving rise to a slow onset disorder comprising both psychiatric and neurological upset. vCJD has probably been acquired by ingestion of meat from animals with bovine spongiform encephalopathy (BSE).

There are no notable oral manifestations of prion disease other than fasciculation of the tongue and oral dysaesthesia-like symptoms in vCJD. While prion material of sporadic CJD is localized to central neural tissue, that of vCJD occurs in peripheral tissues, including lymphoid tissue. There is no evidence that prions have been transmitted as a direct consequence of oral health care; however, the oral tissues of experimental animals can harbour and transmit infectious disease. Until recently it was suggested that all instruments used in the dental treatment of patients with known prion disease should be destroyed after use, but a recent Department of Health risk analysis determined that this was not required, as the chance of prion transmission within the dental health care setting was 10^9 times less than that expected following tonsillectomy. Likewise, it is suggested that the risk of prion transmission via bone substitutes is lower than the risk of death related to lightening or tornados.

It does, however, remain unclear whether prions can contaminate instruments as a consequence of contact with oral fluids during routine dental care, and it is not known whether prions interact with the biofilms of dental unit waterlines.

Reviewing the role of biofilms in infection control

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In the current climate where much media interest has focused on infection control, the association between infections and biofilms is of increasing

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importance. The realization that biofilms can be a source of pathogenic microorganisms has required us to re-evaluate our infection control policies and practice in the clinical setting.

Infection control procedures are designed to prevent the invasion of the body with microorganisms that have the potential to cause disease. In the medical and dental professions there are many opportunities for the spread of infection. Patients and medical and dental staff are all at risk of being exposed to pathogenic bacteria, viruses and fungi as a consequence of dental and medical treatment. The ability of microbes to multiply and spread from person to person (horizontal spread) is of paramount importance. This spread is most obvious in institutions with overcrowding; additionally in treating patients we increase our risks by the production of aerosols from saliva and blood in dental practice and increase the possibility of blood-borne infections by the use of invasive procedures. There are four main sources of infection. (1) Patients with overt infection; these may liberate large numbers of organisms into the environment (e.g. droplets from the mouth). (2) Patients in the prodromal stage of infection, in which organisms are present during an incubation period without evidence of infection (e.g. measles virus, mumps virus, varicella zoster virus). (3) Healthy carriers who have apparently recovered (e.g. from diphtheria, *Streptococcus pyogenes*, hepatitis B virus) and asymptomatic carriers without a clinical history of infection (e.g. hepatitis B virus). (4) The clinical environment, including contaminated objects, equipment and reservoirs of infection (e.g. dental unit water systems, dental appliances and X-ray films).

To minimize the risk of infection, all staff must be aware of procedures required to prevent transmission of infection. Hence, they must follow the policy on decontamination and infection control, be familiar with personal protection requirements and use, and finally what to do in the event of accidents or personal injury.

Infection control in dental technology laboratories

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Dental technicians are trained in a range of skills involved in the fabrication of prostheses used in the mouth and facial region. Items entering the dental laboratory are essentially inert materials that have been in contact with the patient's mouth, saliva, and possibly blood. Appliances leaving the laboratory are then returned to the clinician to be tried/inserted in the patient's mouth.

Relatively little attention has been paid to infection control policy within dental laboratories, perhaps due to perceived and/or actual remoteness from patients, lack of appropriate training, and lack of relevant research. Microbiological audits carried out on equipment, materials, surfaces, etc. in dental technology laboratories have revealed the presence of a range of opportunistic pathogens, predominantly of environmental origin, but occasionally from the oral flora. Impression materials received from the dental surgery, new and old prostheses being handled in the dental laboratory (potential for cross-contamination), the lathe (cross-contamination and aerosol generation), and the plaster trap (heavily contaminated but little risk to personnel) are amongst the contaminated samples that were studied. The infection potential of these microorganisms should be assessed as part of a hazard analysis procedure, and appropriate action taken to minimize exposure of personnel, patient or dentist. However, there is a need for more comprehensive studies before any conclusions can be drawn.

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Review of the treatment aspects of dental unit water systems

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Dental unit water systems (DUWS) have been demonstrated to be heavily contaminated with micro-organisms. As there is currently no European Union Commission guideline applied to DUWS, this project set out to investigate microbial contamination and the application of disinfectants to DUWS for the control of microbial contamination in general dental practice (GDP) in the UK, Denmark, Germany, The Netherlands, Ireland, Greece and Spain.

Microbiological surveys were carried to determine the baseline level of microbial contamination followed by assessment of a range of proprietary products in a reproducible controlled laboratory model. A number of products including Sterilex Ultra, Alpron, Sanosil, Oxygenal and BioBLUE were then selected to be administered according to the manufacturers instructions to the DUWS in GDP. Water samples were again analysed before and after application of the products for microbiological analysis for total viable counts and *Pseudomonas aeruginosa* to assess the microbial load of the DUWS. The microbial load of DUWS in the different countries was up to 4.4×10^4 c.f.u./ml (c.f.u. is colony-forming units). The products Sanosil, HWP Blue, Oxygenal and Dentasept (hydrogen-peroxide-based products) and Alpron were rated as the most successful products in reducing the microbial counts to <200 c.f.u./ml. BioBLUE was rated as the next best product, although Ster4spray was limited in efficacy and Sterilex Ultra was problematic, resulting in clogged and blocked DUWS. Occasional spiking of the microbial load at >200 c.f.u./ml was observed with a number of the products.

Whilst the majority of products achieved the necessary reduction in counts to satisfy the American Dental Association (ADA) criteria of <200 c.f.u./ml, the use of disinfectants is not necessarily a panacea, as practical problems occasionally occurred. The authors would recommend that the dental community monitors the microbial loading of their DUWS and adopts the ADA standard that DUWS water should not exceed 200 c.f.u./ml. This work was supported by the EC (QLK4 –00097-2000).

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