

Combating infections at biomedical implants and devices by antibacterial coatings

Researchers at the University of South Australia describe a number of approaches to the fabrication of thin coatings that confer resistance to colonisation by bacteria. These coatings are intended for application onto biomedical devices to prevent device-related infections caused by bacterial biofilms.

Biomedical devices have become essential parts of the human healthcare system. Over the past two decades, the number of artificial hip and knee implants has increased markedly worldwide. Stents, heart valves, vascular grafts and other organ replacements have been used successfully to save lives and to restore quality of life for many people. Shorter term biomedical devices are various catheters and orthopaedic screws, among others. By far the largest numbers of biomedical products sold, however, are contact lenses. Although contact lenses are often also used as cosmetic accessories to change apparent iris colour, their science is very similar to the science underpinning the development of other biomedical devices. As the Australian population ages, the demand for such biomedical devices continues to increase, and clinicians express their desire for better or longer lasting biomedical devices.

A significant issue in implant surgery and also with short-term biomedical devices is bacterial infection. The colonisation of surfaces of biomedical devices and implants by bacteria can cause infections that pose a health risk to patients, often require re-operation and replacement of the infected device, and incur considerable healthcare costs. The severity of such bacterial infections varies greatly among devices but can be serious or even fatal in some instances. Although early signs of bacterial infection are generally noticed by a wearer of contact lenses, and bacterial contact lens infections are infrequent in the hygienic environment of modern societies, there are no early warning signs of bacterial infections of many other implants and devices, and diagnosis often occurs when a full-blown infection has already caused damage to tissue and organism. Accurate data are hard to come by, for various reasons, but a survey suggests that in Australia and the USA around 4% of hip and knee implants become infected. Re-operation of infected implants has led to the death of elderly patients already



Figure 1. *Eremophila denticulata* subsp. *trisulcata* (Chinnoek), a taxon we identified as having resin with antibacterial activity. Note the glossy waxy appearance of the leaves, which is typical of resinous *Eremophila* species.

weakened by the previous operation or other medical conditions.

Catheters are replaced at frequent intervals to minimise the risk of bacterial infections, but such preventative replacement schedules impose considerable healthcare costs. In the case of implants, the problem cannot be addressed as readily. With improved procedures in sterilisation and operating theatres, the frequency of early-stage infections of implants has decreased significantly. However, delayed infections, occurring many weeks or months after surgery, continue to pose a serious problem. It is thought that these late-stage infections are caused not by the act of surgery but by bacterial spores circulating in the vascular system. Spores landing at an incompletely healed wound site may attach to the implant surface, multiply, and form a biofilm that eventually leads to infection.

Unfortunately, the exocellular matrix, comprising various polysaccharides that provide the slimy feel, protects bacterial colonies very well against antibiotics and the body's innate defence system. Thus, bacterial biofilms attached to solid materials surfaces are much more difficult to eradicate than circulating bacteria; hence the need for surgical removal of a substantial proportion of infected implants. Accordingly, one strategy for reducing the occurrence of infections is to prevent the initial attachment of bacteria to implant and device surfaces. Thus, we are pursuing the development of thin coatings that can be applied to biomedical devices with the aim of providing resistance to bacterial colonisation. Thin antibacterial coatings provide a route to equipping existing devices and implants with improved bacterial resistance while not affecting their other properties, such as the visual clarity of contact lenses or the flexibility of vascular grafts.

Here we describe a number of strategies for the fabrication of antibacterial surface coatings. Given the variety of devices and implants, as well as causative bacteria, a single approach may not be universally successful, and antibacterial strategies may need to be tailored to specific product needs. For some products, such as contact lenses, resistance to the attachment of bacteria is required along with bio-fouling resistance, whereas for hip and knee implants we wish to deter bacteria while enabling close integration of the implant with human tissue. These requirements have to be tackled using different designs of antibacterial coatings.

Approaches to antibacterial coatings

In principle, antibacterial compounds can be used in two ways. One approach is to use a controlled release approach, in which an antibiotic is released from a biomedical device

and intercepts bacteria in the vicinity. By far the most common antibiotic used in this way is silver; several silver-based antibiotic approaches are already well advanced. The scientific consensus is that silver ions, released from silver metal coatings or polymer coatings doped with silver nanoparticles, enter bacteria and affect their biological processes. Other metal ions have also been tested, but adverse effects on human tissue present a concern. In addition, the duration of antibacterial action is limited by loading and release kinetics.

The second approach consists of the fabrication of a surface layer of covalently immobilised antibiotic molecules that prevent bacterial attachment to materials' surfaces. In addition to potentially much longer effectiveness, this approach is favourable when seeking regulatory approval for new devices; if it can be ascertained that the antibiotics are durably anchored on the device surface, one can eliminate concerns about possible adverse effects due to accumulation of antibiotics in body tissues such as brain, liver and spleen.

The largest effort at present on antibacterial coatings at the University of South Australia focuses on the covalent coupling to solid surfaces of molecular layers of novel antibacterial compounds isolated from Australian native plants in the genus *Eremophila* (Fig. 1).¹ With 216 described species, and others recently discovered and yet to be scientifically described, this genus is one of the largest genera of Australian plants, but is not well known, likely due to its prevalence in arid and semi-arid regions. Australian Aboriginal people, however, have used extracts of at least five *Eremophila* species for traditional medicinal purposes such as the treatment of skin sores and sore throats.² The applications for which *Eremophila* extracts were used suggested an antibacterial action, and ethanolic extracts indeed showed *in vitro* antibacterial activity.³ However, the active compounds were not isolated and identified at the time.

Our work first investigated whether antibacterial activity of extracts is confined to the few species used by Aboriginal people. Screening over 70 species, we found that many showed considerable activity against multi-drug resistant strains of key bacteria causing hospital infections.⁴ The reason for selective use of a few species may be that those species are very widespread across much of inland Australia, whereas a high proportion of other *Eremophila* are highly localised. Activity was found to be correlated with the presence of a sticky or waxy resinous coating on leaves and stems, whereas species with hairy, silvery leaves showed no significant activity. Resin layers and hairs represent two alternative strategies against dehydration; in some species, both are employed concurrently.

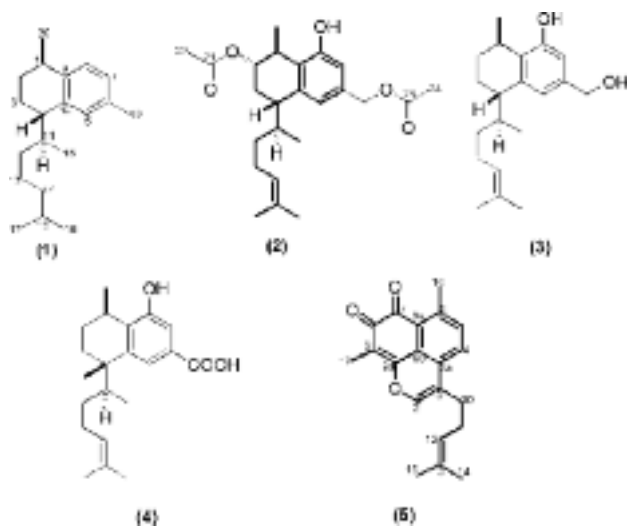


Figure 2. Chemical structures of the serrulatane diterpene skeleton (1), and antibacterially active compounds isolated from *Eremophila neglecta* (2-5). (Reproduced from reference 5 with permission.)

Focusing on three species with active extracts (*E. serrulata*, *E. neglecta* and *E. duttonii*), our research has led to the isolation and structural identification of several active compounds; they all are diterpenes of the serrulatane class. They were found to be active in solution against the key bacteria of interest.^{5,6} Examples of serrulatane diterpenes are shown in Figure 2.

Such serrulatane compounds have been covalently linked to polymer and ceramic substrates via thin (~ 20 nm) adhesive interlayers fabricated by plasma polymerisation.⁷ Covalent anchoring to thin film layers bearing surface amine groups was done in two ways: (1) by carbodiimide-mediated amide formation between a surface amine and a carboxyl group on a suitable serrulatane, such as **4** in Figure 2; (2) via an oxymercury-catalysed condensation of the double bond in the 'tail' of serrulatanes with surface amines. The resultant coatings have been characterised extensively by the surface analytical techniques of X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectroscopy (ToF-SIMS), so that the observed biological responses can be interpreted reliably in terms of documented surface composition and properties. Features such as an aromatic shake-up satellite in the XPS C1s spectrum, arising from the aromatic ring in the diterpene structure, and characteristic ToF-SIMS fragments have enabled verification of the presence of the serrulatanes on the surface, and the success of the intended linking chemistry has even been detected via a fragment containing the serrulatane moiety coupled to an interlayer fragment.

The coatings have been tested for their ability to deter

bacterial adhesion in an in vitro model with *Staphylococcus epidermidis*. Of several coating variants, the best produced greater than 99.8% reduction in bacterial colonisation over four hours compared with a polyallylamine graft coating; representative optical micrographs of samples stained for the presence of live and dead bacteria are shown in Figure 3. Only a few isolated single bacteria managed to attach to the best serrulatane coatings, probably onto coating defects arising from absence of clean-room coating conditions. Those single bacteria did not form clumps and biofilms upon extended culture (up to 48 h), whereas reference surfaces became colonised with extensive bacterial biofilms.

It is also important to ascertain absence of adverse effects on mammalian cells and tissue; such adverse effects occur for example with quaternary amine compounds. Initial cell culture with 3T3 fibroblasts showed good attachment of those cells onto serrulatane-coated surfaces. The in vitro results to date indicate considerable promise for in vivo and clinical studies with these coatings. A provisional patent application has been lodged to protect the novel coatings and their commercial application. Companies have expressed interest in this technology.

An analogous approach has been used to covalently attach furanone molecules onto surfaces. Work at the University of NSW showed that furanones isolated from marine algae were active against medically important bacteria.⁸ We have developed approaches for the covalent immobilisation of furanones onto polymeric surfaces,^{9,10} and the antibacterial effectiveness of surface-immobilised furanones was tested using contact lenses in vitro and in vivo.¹⁰ Contact lenses represent a good test system because bacterial infections can be induced and detected readily, and rapid removal of the infected device is facile.

The approach of using silver nanoparticles in combination with a thin polymeric carrier film has also been successful. By incorporating silver nanoparticles inside a polymer film, direct exposure of the biological environment to solid silver surfaces can be avoided. Our approach has utilised plasma polymer films made from n-heptylamine as polymeric carriers; the advantages of these plasma polymer films are that they are mechanically more robust than the polymeric carriers used in other studies, and their surface is conducive to colonisation by human cell lines and tissue. Antibacterial action is achieved by out-diffusing silver ions. The loading is controlled by the film thickness, and the release rate can be adjusted by the extent of cross-linking and also by the application of a second thin film layer. Tests involving bacterial colonisation in vitro likewise have shown that this strategy leads to considerable reductions in bacterial colonisation.

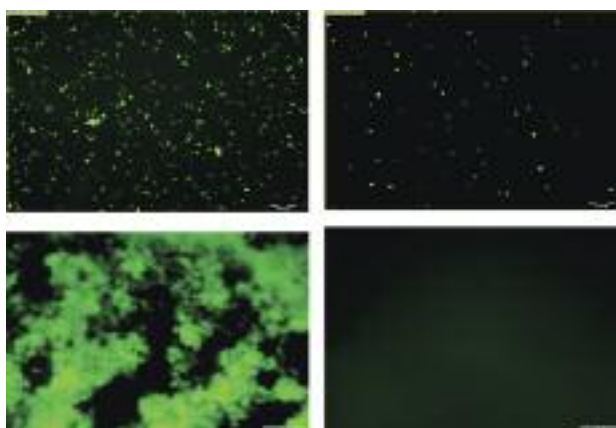


Figure 3. Optical micrographs showing bacterial colonisation of materials surfaces. The bacteria were stained to show up in fluorescent green if alive and in red if dead. Top left: amine plasma polymer surface; bottom left: polyallylamine graft surface; right-hand side: two different serrulatane coatings.

We have also shown that some commercially available, established or experimental antibiotics can be covalently linked to polymer surfaces as molecular layers, and in this manner provide protection against bacterial colonisation *in vitro*. One example is the antibiotic novobiocin, which so far has only been used in solution.

The availability of several alternative antibacterial coating strategies is a great asset because no single coating will be suitable for all applications; with further biological testing we will establish a database for the rational selection of antibacterial coatings for specific applications.

Current directions

Work is continuing on making, characterising and testing coatings, in order to develop optimally efficient coatings. In addition, the research is expanding thanks to a recently awarded National Health and Medical Research Council Development Grant. To date, serrulatane compounds have been sourced by extraction from plant material collected from private gardens or from public land (with permits). It is essential for commercial viability of the technology to investigate the reliability and costs of procuring larger amounts of the compounds. One scenario is the cultivation in plantations of *Eremophila* species that have high loadings of antibacterial compounds, coupled with extraction. We will assess how readily the plants can be propagated and grown using established methods, and, more importantly, the efficiency of extraction. The other scenario is the total chemical synthesis of compounds. A new sub-project has just commenced involving synthesis studies of serrulatane diterpenes, with

PhD student Jessica Cook and Associate Professor M. Perkins (Flinders University). Additional biological testing methodologies are being applied now to test the various coatings in more sophisticated and comprehensive ways, for data collection required for future clinical testing and regulatory approval.



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