Summary

Multidrug resistant surgical infection is a global threat to the practice of surgery. This work is the first to describe a characteristic spectrum of Gram-negative bacteria to infect burn wounds, establish a clinically viable mechanism for passive drug accumulation and “smart release” at any infected soft tissue site. This work subsequently established an entirely novel class of macromolecular antibiotics.

Burn wound infections in different centres were traditionally thought to be caused by differing sets of organisms. However, our metanalysis of standardised bacterial incidence rates confirmed Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanni, Enterobacter spp., Proteus spp. and Escherichia coli as characteristic Gram-negative burn wound pathogens ($F(4,20)=1.1$, $p=0.3797$; $r^2=9.84$). We subsequently identified a common biochemical cascade instigated by these bacteria, Streptococcus and Staphylococcus aureus which is bradykinin-controlled, leading to local vascular permeability enhancement (VPE). The mechanism leads to accumulation of macromolecules around the site of infection. Conventional “small molecule” antibiotics cannot target specifically an infected area-distributing to unintended sites, facilitating toxicity. This work therefore utilised the mechanisms heretofore discussed in custom-designing a prototype polymer-antibiotic conjugate that allows targeting to, and controlled release at, an infected site. I employed the biodegradable, naturally-occurring polymer, dextrin, and a “last-line” antibiotic, colistin, as the first model combination.

Colistin’s toxic multiple amine groups, were eliminated through the formation of peptide bonds, to dextrin, facilitating linkage whilst reducing toxicity. The colistin was therefore “masked” in transit to the target site through chemical bonding and the steric hindrance of a large dextrin chain. The reaction was feasible, predictable and reproducible ($r^2=0.96$, p <0.0001).

Exposed to normal levels of amylase activity (≤100 IU/L), minimally modified low molecular weight dextrin, conjugated to colistin, exhibited an ideal “masking” profile. Ex vivo, this lead compound also rapidly “unmasked” at infected sites. The resulting bioactivity was comparable to the current clinical formulation of colistin (Colimycin) against a clinical library of MDR A. baumanii, K. pneumoniae and E. coli. However the compound suppressed bacterial growth fourfold longer than the equivalent dose of Colimycin (p<0.05).
An ex vivo (n=6) series of human infected burn wound fluid samples confirmed a significantly higher amylase activity around wounds than in plasma. Upon incubation, colistin was easily “unmasked” at the infected site, supporting the notion of locally-triggered, enzymatically-mediated unmasking. An in vivo (n=32) Sprague Dawley rat model reported optimisation of pharmacokinetic parameters (plasma half-life doubled, clearance decreased 6-fold). In contrast to colistin, no clinical toxicity was observed at the highest protocol dose.
This study is the first to establish the pathogens responsible for the majority of Gram-negative burn wound infections. The EPR mechanism is dependent on human bradykinin release in response to non-specific bacterial infection; the accumulated amylase is human, emphasising the applicability of this proof of concept for target antibiotics to most soft tissue

infections, achieving controlled release whilst minimising adverse effect. This approach provides an effective clinical tool for the treatment of surgical soft tissue infection.

Guest lecture: Hunterian Lecture: Multidrug resistant burn wound infection: establishing the causative profile and novel translatable theranostic strategies Mr E Azzopardi (Swansea)