Development and Validation of an *In Vitro* Pharmacokinetic/Pharmacodynamic Model to Test the Antibacterial Efficacy of Antibiotic Polymer Conjugates.

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Running title: PK-PD model for dextrin-colistin conjugates

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ABSTRACT

This study describes the use of a novel, two-compartment, static, dialysis bag model to study the release, diffusion and antibacterial activity of a novel, bioresponsive dextrin-colistin polymer conjugate against multidrug resistant (MDR) wild-type Acinetobacter baumannii. In this model, colistin sulfate, at its MIC, produced a rapid and extensive drop in viable bacterial counts (<2 log₁₀ CFU/mL at 4 h), however, a marked recovery was observed thereafter, with regrowth equivalent to that of control by 48 h. In contrast, dextrin-colistin conjugate, at its MIC, suppressed bacterial growth for up to 48 h, with 3 log₁₀ CFU/mL lower bacterial counts after 48 h, compared to control. Doubling the concentration of dextrin-colistin conjugate (to 2 x MIC) led to an initial bacterial killing of 3 log₁₀ CFU/mL at 8 h, with a similar regrowth profile to 1 x MIC treatment thereafter. The addition of colistin sulfate (1 x MIC) to dextrin-colistin conjugate (1 x MIC) resulted in undetectable bacterial counts after 4 h, followed by suppressed bacterial growth (3.5 log₁₀ CFU/mL lower than control at 48 h). Incubation of dextrin-colistin conjugates with infected wound exudate from a series of burn patients (n=6) revealed an increasing concentration of unmasked colistin in the OC over time (up to 86.3% of initial dose at 48 h), confirming that colistin would be liberated from the conjugate by endogenous α-amylase within the wound environment. These studies confirm the utility of this model system to simulate the pharmacokinetics of colistin formation in humans administered dextrin-colistin conjugates and further supports the development of antibiotic polymer conjugates in the treatment of MDR infections.
Introduction

In an attempt to meet the challenge of the global epidemic of antibiotic-resistant infections, treatment strategies are increasingly employing nanomedicine-based approaches, which have been used in the treatment of a number of human diseases with some considerable success (1, 2). The adoption of these approaches as antimicrobial therapies has seen the pre-clinical development of novel agents including antibiotic-bearing nanoparticles, liposomes, dendrimers and polymer therapeutics (3, 4); agents which may offer potential benefits over conventional systemic delivery in minimizing toxicity and overcoming drug resistance by affording ‘selective targeting’ of systemically-delivered antibiotics to sites of infection and inflammation, thus maximizing local bioavailability of the chemotherapeutic agent (3, 5). The employment of ‘polymer therapeutics’, in which an antibiotic is covalently attached to a water-soluble polymer, may afford a number of important practical advantages, including increased solubility and bioavailability, decreased toxicity and a chemically modifiable controlled release system which can be truly “customized” (2, 4, 6).

In this respect, we have recently described a ‘nanoantibiotic’ polymer therapeutic, based on dextrin conjugated to colistin, whereby colistin is controllably released from the conjugate by amylase-triggered degradation of dextrin, according to the polymer masked-unmasked protein therapy (PUMPT) principle (7), and in so doing, reinstates the antibiotic activity of colistin (8, 9). These dextrin-colistin conjugates demonstrated antibacterial activity across a range of Gram-negative bacteria that was comparable to colistin methanesulfonate (CMS), but exhibited reduced *in vitro* and *in vivo* toxicity and prolonged plasma half-life.

In the preliminary design and development of any novel chemical entity it is pivotally important to understand its pharmacokinetic-pharmacodynamic (PK-PD) profile, before moving to *in vivo* efficacy studies. This is particularly important in nanoantibiotic delivery systems as the incorporation of conventional small molecules into nano-sized structures is associated with
substantially altered PK-PD properties from the original antibiotic (10, 11). However, the complex release or formation of drug from nanoantibiotics renders conventional PK-PD models inappropriate in the preclinical development of these controlled release systems (10, 11). Conventional single-compartment static models, whilst extensively utilized to study the effect of fixed antibiotic doses on fixed bacterial loads (12, 13), are typically only viable for \( \leq 24 \) h (14, 15), and are of limited value in investigating drug release and real-time antimicrobial effects of controlled release drugs (16, 17). Single compartment ‘time to kill’ (TTK) models are also unable to prevent denaturation of the macromolecule’s activating enzyme (e.g. \( \alpha \)-amylase) by artificial media used to sustain bacterial growth (16, 17). Multiple chamber dynamic models may be used to produce concentration gradients, although require considerable technical expertise, specialist software and are associated with high costs (18). In the development and screening of nanoantibiotics, a need, therefore, exists for a suitable \textit{in vitro} model to define the PK-PD profiles and optimize the characteristics of these novel agents prior to \textit{in vivo} testing.

This study employed dextrin-colistin conjugates as a prototypical nanoantibiotic, to develop a model system which would afford the opportunity to study the \textit{in vitro} degradation, diffusion and activation of the conjugate, both alone and in the presence of amylase or infected wound fluid. The study sought to define an \textit{in vitro} model in which the PK-PD parameters of biodegradable antibiotic polymer conjugates could be reliably assessed.

\textbf{MATERIALS AND METHODS}

\textbf{Materials.} Colistin sulfate, ethanol, human salivary \( \alpha \)-amylase, 4-dimethylaminopyridine (DMAP), anhydrous N,N-dimethylformamide (DMF), succinic anhydride, diethyl ether, type 1 dextrin from corn starch (mol wt \( \sim 8,100 \) g/mol), carbonic anhydrase (29,000 g/mol), were purchased from Sigma Aldrich (Poole, UK). Sodium hydrogen orthophosphate, potassium
dihydrogen orthophosphate, potassium chloride, and sodium hydroxide (NaOH), copper (II) sulfate pentahydrate, bicinchoninic acid (BCA) assay kit and 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) were purchased from Thermo Fisher Scientific (Loughborough, UK). Sterile 0.9% sodium chloride solution was obtained from Baxter (Berkshire, UK). N-hydroxysulfosuccinimide (sulfo-NHS) was purchased from Fluka (Buchs, Switzerland). Trypticase soy agar (TSA), trypticase soy broth (TSB) and cation adjusted Mueller Hinton agar (CAMHB, lot 43145) was purchased from Oxoid (Basingstoke, UK). Polyvinylidene fluoride sterile membrane syringe filters (0.22 μm) were from Elkay (Basingstoke, UK). Tegaderm® semi-permeable dressings were purchased from 3M Healthcare (Neuss, Germany). Sterile graduated plastic syringes were from BD plastipak (Madrid, Spain). Phadebas® amylase test was from Magle Life Sciences (Lund, Sweden) and MaxSignal® colistin enzyme-linked immunosorbent assay (ELISA) kit was purchased from Bioo Scientific Corp. (Austin, USA). All chemicals were of analytical grade.

**Escherichia coli** (E. coli) National Collection of Type Cultures (NCTC) 10418 and **Acinetobacter baumannii** (A. baumannii) 7789 (v19) from clinically infected wounds (19)) were donated by Dr Robin Howe (Public Health Wales Microbiology Laboratory, University Hospital of Wales, Cardiff, UK). Sensitivities of these strains have been reported previously (19). MIC values of A. baumannii 7789 have been previously reported as 0.0625 and 8 μg/mL for colistin sulfate and dextrin-colistin conjugate (+ amylase), respectively (20).

**Synthesis and characterization of dextrin-colistin conjugates.** Dextrin-colistin conjugates were synthesized and characterized as previously described (8, 9). The dextrin-colistin conjugate used in these studies contained dextrin with 1.0 mol% succinoylation and had a mol wt of approximately 10,300 g/mol (gel permeation chromatography (GPC) with pullulan standards) and a colistin content of 11.2% w/w (BCA assay) with < 3% free colistin (fast protein liquid chromatography (FPLC)).
**In vitro PK-PD model.** Unmasking of dextrin-colistin conjugates was simulated using a two-compartment static, ‘dialysis bag’ model under sink conditions (total volume: 20 mL, inner compartment volume: 5 mL), as shown schematically in Fig. 1. Dialysis membrane (10,000 g/mol MWCO) was pre-soaked for 15-30 min in distilled water (dH2O), secured with dialysis clips and suspended from an injection port to separate the inner compartment (IC) from the outer compartment (OC) in a sterilized 25 mL beaker sealed with sterile medical grade polyurethane membrane (Tegaderm®). The model system was prepared under aseptic conditions in a class 2 laminar air flow cabinet, and transferred to a shaking incubator set at 37°C in ambient air and constant orbital agitation at 70 RPM for 48 h.

**Validation of in vitro PK-PD model.** To validate the ability of the dialysis membrane to retain intact dextrin-colistin conjugate in the IC while permitting unrestricted diffusion of unmasked colistin to the OC, protein content in both compartments was measured over time. Briefly, the IC was spiked with dextrin-colistin conjugate (equivalent to 10 µg/mL colistin) in PBS (pH 7.4, 37°C) in the absence and presence of α-amylase (100 IU/L), while the OC contained sterile PBS (15 mL). The sealed beaker was incubated as described previously. Samples (150 µL) were extracted from each compartment at various intervals (0, 4, 8, 12, 24, 36, 48 h) using sterile, medical-grade single-use vascular catheters, and immediately frozen on dry ice and stored at -20°C prior to analysis. Protein content was determined using a standard BCA assay and antimicrobial susceptibility of *E. coli* NCTC 10418 isolates was assessed using an MIC assay (see below). At the end of the 48 h incubation, FPLC was used to estimate the proportion of ‘unmasked’ colistin in both compartments by calculating the area under the chromatographic curve. At the end of the experiment, integrity of the dialysis membrane to α-amylase was confirmed using carbonic anhydrase as a molecular weight marker. Contents of both IC and OC were removed. The dialysis membrane was flushed with several volumes of sterile dH2O and then re-suspended in sterile PBS as described.
previously. The IC was spiked with carbonic anhydrase (2 µg/mL) in PBS. After a further 17 h incubation with PBS in the OC, the ratio of carbonic anhydrase in both compartments was quantified by UV/vis spectrophotometry (n=3).

**Susceptibility testing.** Antimicrobial activity in the OC was measured in *E. coli* NCTC 10418 isolates in a standard MIC assay (21). Isolates were grown overnight in TSB and then diluted in sterile PBS to an optical density (OD625) of between 0.08 and 0.10 (equivalent to 0.5 McFarland standard; approximately 10^8 CFU/mL). Serial two-fold dilutions were prepared for each sample in flat-bottom 96-well microtiter plates (100 µL per well). The diluted bacterial cultures were then diluted ten-fold in CAMHB, and 5 µL was added to each well of the microtiter plate. Plates were incubated at 37°C in ambient air for 18-20 h and the lowest concentration at which there was no visible growth was taken as the MIC. In addition, on each plate, triplicate wells were prepared containing no antibiotic and no bacterial inoculum as growth and sterility controls, respectively.

**Time to kill (TTK) assay.** A modified TTK assay was used to investigate the concentration- and time-dependent antimicrobial activity of colistin sulfate, dextrin-colistin conjugate, and a mixture of colistin sulfate + dextrin-colistin conjugate against *A. baumannii* 7789. In these experiments, the OC contained approximately Log_{10} 5 CFU/mL of *A. baumannii* 7789 in MHB and the IC contained α-amylase (100 IU/L) in sterile PBS in combination with either: a) colistin sulfate (0.25 µg/mL, 1 x MIC); b) dextrin-colistin conjugate (32 µg/mL colistin equiv., 1 x MIC); c) dextrin-colistin conjugate (64 µg/mL colistin equiv., 2 x MIC); or d) a mixture of colistin sulfate (0.25 µg/mL, 1 x MIC) + dextrin-colistin conjugate (32 µg/mL colistin equiv., 1 x MIC). Colony counts (CFU/mL), from samples taken as described previously, were performed in triplicate according to the method of Miles *et al.* (22). Briefly, samples (50 µL) were diluted 10-fold in PBS and centrifuged. Pellets were resuspended in an
equivalent volume of fresh PBS. Serial 10-fold dilutions were performed in triplicate across a 96-well plate. Samples (5 µL) from each dilution were dropped onto the surface of the dried TSA plates in triplicate. TSA plates were left undisturbed for 30 min, inverted, and incubated for 18-20 h (37°C in ambient air) then visually inspected for growth. The drop-position displaying the highest number of full-size discreet colonies (range 2-20 colonies) was quantified and multiplied by the dilution factor. The resulting colony count (CFU/mL) was plotted versus time to produce a TTK curve. Growth and sterility controls in the absence of antibiotic or A. baumannii 7789, respectively, were performed in triplicate.

**Ex vivo sample collection.** Following research ethics committee approval and informed consent, wound fluid samples were collected from adult infected burn wounds (n=6) being treated at the Welsh Burns Centre (Swansea, UK). Burn wound infection was diagnosed according to the American Burn Association diagnostic criteria (23). Patients with recorded pancreatic or salivary disease were excluded. After dressing removal, wounds were lightly washed with sterile 0.9% sodium chloride solution and a sterile Teak® graft board used to collect the exudate. Samples were then transferred to a sterile syringe, sealed, frozen on dry ice and stored at -80°C until required. Samples were centrifuged (x 15,000 g for 5 min at 4°C) then the α-amylase content was determined in triplicate using a Phadebas® amylase test according to the manufacturer’s instructions.

**In vitro modeling of dextrin-colistin conjugate ‘unmasking’ in infected wound fluid.** The same PK-PD setup was used to evaluate the feasibility of dextrin-colistin conjugate unmasking in infected burn wound samples. Each wound fluid sample was diluted 2-fold in PBS to make up the IC volume (5 mL) and dextrin-colistin conjugate (64 µg/mL colistin equiv., 2 x MIC) was added. For each wound sample the procedure was repeated in triplicate. Total colistin content in the OC was quantified by ELISA according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microtiter plate reader and these
values were used to calculate colistin concentration in the OC (mean ± SD (n=3)) according to calibration curve (assay detection range 0.5-50 ng/mL). The proportion of unmasked conjugate in the OC was calculated using FPLC, as previously described.

RESULTS

Method validation. Protein concentration in the IC decreased in a logarithmic fashion over time, while protein concentration in the OC increased reciprocally (Fig. 2). A corresponding increase in antimicrobial activity of the OC over the 48 h incubation was also observed (Fig. 2). FPLC demonstrated that, at 48 h, significant unmasking had occurred (p<0.05), with unmasked conjugate accounting for ~68% of total colistin content in the system (Fig. 3). At 48 h there was no significant difference in the distribution of unmasked conjugate between the two compartments (p>0.05). Correspondingly, there was a significantly higher proportion of high molecular weight (masked) dextrin-colistin conjugate in the IC (~80%, p<0.05), compared to the OC (Fig. 3b). When the experiment was conducted using dextrin-colistin conjugate in the absence of α-amylase, the OC protein content was <10% after 17 h. Membrane integrity testing confirmed the presence of carbonic anhydrase in the OC was always below the lower limit of quantification (data not shown).

PK-PD modeling against A. baumannii 7789 isolates. Colistin sulfate, at its MIC (0.0625 μg/mL) showed rapid initial killing, and viable bacterial counts were below the assay's lower limit of quantification at 4 h. However, early and significant bacterial re-growth, to the level seen for control, was observed from 8 h onwards (Fig. 4). Dextrin-colistin conjugate was bactericidal at 2 x MIC, where maximum reduction in viable bacterial counts (~ Log_{10} 3 CFU/mL) occurred at 8 h. Thereafter, re-growth was slower than in the presence of colistin sulfate. In contrast, the colistin sulfate + dextrin-colistin conjugate combination retained optimal characteristics of both components, with rapid initial bacterial killing, maximum
reduction in viable bacterial counts at 4 h and sustained reduction of viable bacteria throughout the experiment. The control growth curves confirmed that bacterial growth could be maintained under the experimental conditions, while sterility controls verified that sterility conditions were maintained throughout. Culture onto TSA plates at the end of each experiment confirmed the presence of a homogenous culture.

α-Amylase activity in clinical samples. Wound fluid was collected from a total of 6 patients (Table 1) with partial thickness skin burns. Total protein content in the supernatant was 50.7 ± 15.1 mg/mL (mean ± SD). These studies revealed that α-amylase activity was significantly increased in infected wound fluid (408.4 ± 168.3 IU/L, mean ± SD) compared to patient-matched serum (60.0 ± 25.3 IU/L, mean ± SD) (p<0.05) (Fig. 5).

Unmasking of dextrin-colistin conjugate in infected wound fluid. A time-dependent increase of colistin concentration in the OC was observed in all cases (Fig. 6). Similarly, concentration of colistin in the OC also increased in the presence of higher α-amylase activity, equating to a mean OC colistin content of 68.1 to 86.3% of the theoretical concentration of the original dose concentration at equilibrium (16 µg/mL) at 48 h.

DISCUSSION

We hypothesized that a static, two-compartment dialysis bag model under infinite sink conditions would offer a simple and cost-effective method for the initial PK-PD characterization of biodegradable antibiotic polymer conjugates. Using dextrin-colistin conjugate, we have shown that the model can be employed to study degradation, diffusion and activity across a biological membrane. Moreover, we have demonstrated how these processes may be modified by local enzyme activity at sites of infection.

The use of a two-compartment system permitted the discrete sampling and detailed analysis of the individual compartments over time. Furthermore, this model enabled the
composition of the two compartments to be modified to represent the environmental conditions of the disease of interest, as demonstrated here using simulated and real biological fluids containing physiological concentrations of \( \alpha \)-amylase. The \textit{ex vivo} model described here could easily be adapted to also allow modeling of disease- and target site-specific levels of enzymatic activity in the assessment of enzyme-triggered pharmacokinetics and pharmacodynamics using alternative biological fluids (e.g. wound fluid or bronchoalveolar lavage fluid). The model utilized Spectra/POR \( 7^\circledR \) membrane to prevent non-specific drug binding to the membrane and heavy metal contamination (24-26). The MWCO of the dialysis membrane serves as a size exclusion barrier and can be selected according to the molecular weight of the drug carrier, antibiotic or activating enzyme. Thus, the dialysis membrane can also effectively prevent premature denaturation of the activating enzyme by artificial media in the OC. The dextrin-colistin conjugates studied here were 'activated' by \( \alpha \)-amylase-mediated degradation of dextrin, resulting in reduction of its molecular weight and diffusion of the unmasked conjugate along a concentration gradient to the OC (Fig. 1), where bioactivity could be assayed under various conditions. In this regard, an IC:OC ratio of 1:4 v/v, and total volume of 20 mL in the system, maintained infinite sink conditions, provided a concentration gradient and prevented OC saturation with unmasked conjugate. The infinite sink strategy presented a convenient, cost-effective means of controlling localized drug concentrations (18, 27), and monitoring drug activation in the IC and the resulting bioactivity in the OC. Considering the controlled release nature of the dextrin-colistin conjugate, it was essential to provide an incubation environment that resembled \textit{in vivo} conditions, whilst ensuring bacterial viability. Therefore, 37\(^\circ\)C was selected to mimic \textit{in vivo} conditions; constant agitation reduced the risk of boundary-layer effects at the dialysis membrane (27) and contributed to the maintenance of bacterial growth. Indeed, this two-compartment model demonstrated colistin
sulfate's rapid bacterial killing and subsequent bacterial re-growth observed in previously validated one-compartment models (28-30).

The ability of the dialysis membrane to retain the intact conjugate within the IC until it is 'unmasked' was confirmed during validation, where, in the absence of α-amylase, OC protein content remained <10% at 17 h. The addition of α-amylase, led to an increase in protein content and antimicrobial activity of the OC, which was mirrored by decreased bacterial counts in the TTK study, and were in agreement with previous in vitro physicochemical data (8, 9).

Analysis of bacterial counts in the presence of colistin sulfate was interesting and showed that bacterial recovery occurred earlier than the shortest currently recommended dosing interval for colistin (31, 32), demonstrating the clinical benefit that these models may offer. In contrast, with dextrin-colistin conjugate, the recovery of bacterial counts was significantly delayed, presumably due to the sustained release of colistin from dextrin-colistin conjugates by α-amylase. Since colistin is a 'concentration-dependent' antibiotic (33) with a modest post-antibiotic effect (34), the ability of the polymer conjugates to prolong exposure of bacteria to colistin may be more beneficial to antibacterial efficacy than achieving high peak concentrations (25, 35, 36).

Several concentration-dependent antibiotics, including azithromycin, amikacin and ciprofloxacin, have been incorporated into controlled release polymeric carriers, such as nanoparticles and liposomes (37-39), which could readily be tested in our in vitro system. For instance, Wang used a similar two-compartment model to characterize the in vitro release kinetics of colistin sulfate-loaded liposomes (40). In this study, a combination of colistin sulfate + dextrin-colistin conjugate provided a rapid decline in viable counts, followed by sustained suppression of bacterial growth rate. The conjugate 'unmasking' by α-amylase was shown to
be time-dependent, meaning that, in clinical use, administration of an initial dose of conventional antibiotic may be required when treating acute disease. In addition to improving the therapeutic efficacy of nanoformulated drugs, incorporating antibiotics into nano-sized structures also converts conventional "small molecule" antibiotics into macromolecules, which may benefit from passive, size-based targeting and accumulation at sites of inflammation/infection via the enhanced permeability and retention (EPR) effect (4, 41). A potential limitation of this model is its inability to replicate the potential benefits of the EPR effect, representing an avenue for future development.

Modification of the IC to incubate dextrin-colistin conjugate directly in \textit{ex vivo} infected wound fluid represented an adaptation of the PK-PD model to a higher level of complexity to enable \textit{ex vivo} testing. In these studies, \(\alpha\)-amylase concentration in burn wound fluid was markedly higher than in plasma. These results cannot be simply explained by the effects of thermal injury, as the mean time of sample collection was more than 2 weeks post-burn, when vascular permeability and interstitial fluid leakage have largely subsided (42, 43). Incubation of dextrin-colistin conjugates in infected wound fluid demonstrated that colistin would be readily released from the conjugate locally at infected sites due to local \(\alpha\)-amylase activity. Conjugate unmasking rate (68.1 to 86.2% at 48 h) compared favorably to published values for CMS, for which conversion to colistin in \textit{ex vivo} human plasma samples after 48 h was \(\sim\)60% (44).

Recent advances in the design and development of nanoantibiotics, with complex drug release profiles, warrants the establishment of a novel PK-PD model for monitoring their drug release and antibiotic efficacy. This two-compartment model system permits increasing levels of complexity to be built in, providing a versatile tool to describe the effect of bioresponsive dextrin-colistin conjugates by characterization of \textit{in vitro} drug release (in simulated and \textit{ex vivo} biological fluids) and bacterial killing over time. It is anticipated that these results will
assist in designing optimal dosing strategies for dextrin-colistin conjugates \textit{in vivo}.

\section*{ACKNOWLEDGEMENTS}

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**Figure legends**

Fig. 1: Schematic illustration of (a) the two-compartment static dialysis bag PK-PD model under infinite sink conditions and (b) the hypothesized distribution of dextrin-colistin conjugates.

Fig. 2: Distribution and antimicrobial activity of dextrin-colistin conjugates in a two-compartment dialysis bag model under infinite sink conditions. Panel (a) shows protein concentration in the IC and OC over time (mean ± SD, n=3); panel (b) shows the corresponding MIC for each time point as a measure of antimicrobial susceptibility (mode, n=3).

Fig. 3: Distribution of masked and unmasked dextrin-colistin conjugate at 48 h in a two-compartment dialysis bag model under infinite sink conditions. Panel (a) shows the relative amounts of masked and unmasked dextrin-colistin conjugates in the system at 48 h; (b) shows the distribution of masked and unmasked dextrin-colistin conjugate in each compartment at 48 h. Data shown represents mean ± SD (n=3). *Indicates statistical significance (p < 0.05).

Fig. 4: TTK curves against *A. baumannii* 7789 clinical isolate. Panel (a) shows bacterial viability count following a challenge with colistin sulfate or dextrin-colistin conjugate in the presence of α-amylase (100 IU/L). Panel (b) reports bacterial viability count following a challenge with dextrin-colistin conjugate at 2 x MIC or colistin sulfate + dextrin-colistin conjugate in the presence of α-amylase (100 IU/L). Data shown represents mean CFU values, n=3. Where ● = growth control; ◇ = colistin sulfate (1 x MIC); ▼ = dextrin-colistin conjugate (1 x MIC); × = dextrin-colistin (2 x MIC); and □ = colistin sulfate (1 x MIC) + dextrin-colistin conjugate (1 x MIC). Lower limit of quantification: Log10 2 CFU/mL.

Fig. 5: Patient-matched α-amylase activity in infected wound fluid (Phadebas® assay) (mean ±
SD, n=3) and plasma from clinical reports. The average data set represents mean $\alpha$-amylase activity in wound fluid and plasma samples (mean ± SD, n=6). *Indicates a statistically significant difference (t-test, $p < 0.05$).

Fig. 6: Estimation of unmasked colistin release after incubation of masked conjugate in infected burn wound fluid. Panel (a) reports OC colistin content over time. Data shown represents mean ± SD, n=3. Panel (b) shows wound fluid $\alpha$-amylase concentration determined by the Phadebas® assay and the final $\alpha$-amylase activity in the IC after dilution. aValues represent mean ± SD, n=3. bValues represent mean unmasked dextrin-colistin conjugate (95% confidence interval) by FPLC.
# TABLE 1 Basic demographic characteristics of patients included in this study

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<tr>
<th>Parameter</th>
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<td>Aetiology (n)</td>
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<tr>
<td>Mean age (range)</td>
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<tr>
<td>Total body surface area burnt (range)</td>
<td>5-90 %</td>
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<tr>
<td>Mean weight on admission (range)</td>
<td>80.6 kg (65.0-105.4)</td>
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<tr>
<td>Predominant burn depth (n)</td>
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<tr>
<td>Mean time of sample collection post-burn (SD)</td>
<td>17 days (±6)</td>
</tr>
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</table>
REFERENCES


with alginate oligosaccharides able to potentiate the action of selected antibiotics.


26. Li J, Milne RW, Nation RL, Turnidge JD, Smeaton TC, Coulthard K. 2003. Use of high-performance liquid chromatography to study the pharmacokinetics of colistin...


FIG 1 Schematic illustration of (a) the two-compartment ‘static’ dialysis bag PK-PD model under infinite sink conditions and (b) the hypothesized distribution of dextrin-colistin conjugates.
FIG 2 Distribution and antimicrobial activity of dextrin-colistin conjugates in a two-compartment dialysis bag model under infinite sink conditions. Panel (a) shows protein concentration in the IC and OC over time (mean ± SD, n=3); panel (b) shows the corresponding MIC for each time point as a measure of antimicrobial susceptibility (mode, n=3).
FIG 3 Distribution of masked and unmasked dextrin-colistin conjugate at 48 h in a two-compartment dialysis bag model under infinite sink conditions. Panel (a) shows the relative amounts of masked and unmasked dextrin-colistin conjugates in the system at 48 h; (b) shows the distribution of masked and unmasked dextrin-colistin conjugate in each compartment at 48 h. Data shown represents mean ± SD (n=3). *Indicates statistical significance (p < 0.05).
FIG 4 TTK curves against *A. baumannii* 7789 clinical isolate. Panel (a) shows bacterial viability count following a challenge with colistin sulfate or dextrin-colistin conjugate in the presence of α-amylase (100 IU/L). Panel (b) reports bacterial viability count following a challenge with dextrin-colistin conjugate at 2 x MIC or colistin sulfate + dextrin-colistin conjugate in the presence of α-amylase (100 IU/L). Data shown represents mean CFU values, *n=3*. Where ● = growth control; ◇ = colistin sulfate (1 x MIC); ▼ = dextrin-colistin conjugate (1 x MIC); × = dextrin-colistin (2 x MIC); and □ = colistin sulfate (1 x MIC) + dextrin-colistin conjugate (1 x MIC). Lower limit of quantification: Log_{10} 2 CFU/mL.
FIG 5 Patient-matched α-amylase activity in infected wound fluid (Phadebas® assay) (mean ± SD, n=3) and plasma from clinical reports. The average data set represents mean amylase activity in wound fluid and plasma samples (mean ± SD, n=5). *Indicates a statistically significant difference (t-test, p < 0.05).
FIG 6 Quantification of unmasked colistin release after incubation of masked conjugate in infected burn wound fluid. Panel (a) reports colistin content in the OC over time for 3 patient samples. Data shown represents mean ± SD, n=3. Panel (b) shows wound fluid α-amylase concentration determined by the Phadebas® assay and the final α-amylase activity in the IC after dilution. aValues represent mean ± SD, n=3. bValues represent mean unmasked dextrin-colistin conjugate (95% confidence interval, n=3) by FPLC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amylase activity in undiluted wound fluida (IU/L)</th>
<th>Amylase activity in IC (IU/L)</th>
<th>Colistin concentration in OC at 48 ha (µg/mL)</th>
<th>Calculated unmasked conjugate in OCb (%)</th>
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<tbody>
<tr>
<td>A</td>
<td>710 ± 26</td>
<td>71</td>
<td>13.8 ± 0.7</td>
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<td>B</td>
<td>322 ± 13</td>
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<td>13.2 ± 0.5</td>
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<td>256 ± 3.9</td>
<td>25.6</td>
<td>10.9 ± 1.3</td>
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