Chelated iron sources are inhibitors of *Pseudomonas aeruginosa* biofilms and distribute efficiently in an *in vitro* model of drug delivery to the human lung

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Abstract
Aims: To determine whether chelated sources of ferric iron were efficient inhibitors of biofilm formation in *Pseudomonas aeruginosa* and might be suitable for drug delivery to the lungs of cystic fibrosis (CF) patients via nebulization.

Methods and Results: The response of *P. aeruginosa* biofilms to elevated iron concentrations in the form of eight structurally varied iron chelators in a microtitre plate assay for biofilm production was examined in the lab. Among these iron chelates, picolinic acid and acetohydroxamic acid-chelated iron were able to effectively thwart biofilm production in *P. aeruginosa* PA14 and in 20 clinical isolates of *P. aeruginosa* from a local hospital. The chelated iron sources showed excellent distribution in an Anderson cascade impactor model of particle size distribution in the human lung.

Conclusions: Ferric picolinate and ferric acetohydroxamate are effective antibiofilm compounds against both lab and clinical strains of *P. aeruginosa* and are readily nebulized into particles of suitable size for lung delivery.

Significance and Impact of the Study: The data herein serve both to solidify the growing base of literature correlating high iron levels with biofilm inhibition in *P. aeruginosa* and to highlight the potential of these chelators as nebulized agents to combat biofilms of *P. aeruginosa* in CF patients.

Introduction

*Pseudomonas aeruginosa* is a ubiquitous, Gram-negative human pathogen responsible for a variety of infections in immunocompromised humans and is the chief aetiological agent of morbidity and mortality in cystic fibrosis (CF) (van Delden and Iglewski 1998; Costerton et al. 1999; Parsek and Singh 2003; Banin et al. 2005). Treatment of *P. aeruginosa* infections is often complicated by the ability of this organism to form hearty, surface-attached communities known as biofilms. These differentiated bacteria possess vastly enhanced antibiotic resistance compared with their planktonic brethren. A mounting body of evidence (including biofilm-type bacterial aggregation in *ex vivo* CF mucus preparations in addition to the presence and correct ratios of quorum sensing signals to indicate biofilm formation in CF sputum) suggests that *P. aeruginosa* utilizes the biofilm phenotype to persist in the chronic, inevitably deadly infections that are the hallmark of the CF lung (Singh et al. 2000; Matsui et al. 2006).

Biofilms of *P. aeruginosa* and other species have quite a different survival strategy than their free-floating kin, evidenced by their generally slower growth patterns and vast changes in the expression levels of various proteins. For example, *P. aeruginosa* biofilms have at least sixfold up- or downregulation of more than 800 genes compared with planktonic levels during the course of their differentiation into mature biofilms; this is well over 50% of this organism’s genome (Sauer et al. 2002). Similar changes occur in other bacterial species like *Escherichia coli* and *Staphylococcus aureus* during their differentiation into...
biofilm structures (Beenken et al. 2004; Ren et al. 2004). Because of the evident differences in antibiotic susceptibility, protein expression and morphology that biofilms present, it has been often hypothesized that biofilm bacteria possess an entirely different and specific set of drug targets not present in planktonic microflora. Indeed, the trend towards biofilm-specific antimicrobial agents has produced numerous lead compounds (Manefield et al. 2004; Geske et al. 2002; Olsen et al. 2002; Hentzer and Givskov 2003; Gillis and Iglewski 2004; Glansdorp et al. 2004; Geske et al. 2005; Musk and Hergenrother 2006; Wolf-Rainer 2006; Huigens et al. 2007).

Recently, iron has arisen as a point of focus in the biofilm literature. Singh et al. discovered that treating *P. aeruginosa* with lactoferrin, a ferric iron-chelating protein, prevented the bacteria from forming biofilms (Singh et al. 2002). It was later discovered that treatment with supraphysiological iron concentrations also prevented biofilm formation in *P. aeruginosa* and caused detachment and clearance of preformed biofilms in flow-chamber experiments (Musk et al. 2005). This effect of high iron concentrations inhibiting biofilm formation was also recently observed in a different strain of *P. aeruginosa* (Yang et al. 2007). These authors show that high levels of iron suppress the release of DNA, an important structural component of biofilms. As DNA release in *P. aeruginosa* biofilms is (at least partially) controlled through the *pqs* operon, it was suggested that iron exerts its anti-biofilm effect through repression of DNA release via the *pqs* operon (Yang et al. 2007).

Thus, it appears that *P. aeruginosa* biofilm formation is operative across a narrow range of iron concentrations (∼1–100 μmol l⁻¹), above and below which the organism can grow only in a planktonic state. Biofilm sensitivity to iron has also been demonstrated in *S. aureus* (Johnson et al. 2005) and *Streptococcus* spp. (Berlutt et al. 2004; Martinthon et al. 2005).

Because biofilms play an important role in lung infections in CF patients, efficient inhibitors of *P. aeruginosa* biofilm formation hold considerable promise as therapeutic agents. Furthermore, the human lung has unique potential for selective delivery of anti-biofilm drugs directly to the site of a biofilm infection. Indeed, nebulized pharmaceuticals are used by virtually all CF patients who undergo medical treatments. These include Pulmozyme™, a recombinant form of DNAse I that thins mucosal secretions (Shak 1995; Suri 2005), and TOBIT™, an inhaled form of the antibiotic tobramycin to treat *P. aeruginosa* infections (Geller et al. 2002; Cheer et al. 2003; Murphy et al. 2004). These treatments allow for comparatively massive concentrations of antibiotic or other pharmacological agent to be administered to the lungs, concentrations that would be impractical or even deleterious if given systemically. Because of this ability, even therapeutic agents of only moderate potency stand an excellent chance of achieving clinically relevant concentrations through direct delivery to the lungs by nebulization. For example, a standard 300 mg dose of TOBIT™ produces a peak sputum concentration in the lungs of approximately 1200 μg ml⁻¹ and a peak serum concentration of 0.9 μg ml⁻¹, while a serum concentration of only 12 μg ml⁻¹ can cause serious complications owing to cochlear and renal toxicity (Geller et al. 2002).

Intrigued by a putative role for elevated iron concentrations in the treatment of CF, we evaluated the anti-biofilm properties of iron (chelated by a number of commercially available and in some cases clinically utilized iron chelators) against *P. aeruginosa* PA14. In addition, we probed the viability of using these chelated iron forms as nebulized drugs for the treatment of CF by examining their particle size distribution profiles in an Andersen cascade impactor model. The most potent chelated iron sources were then evaluated for anti-biofilm activity in a battery of clinical *P. aeruginosa* strains isolated from the sputum of CF patients.

**Materials and Methods**

**Bacterial strains and sources**

*Pseudomonas aeruginosa* PA14: This strain was a kind gift from D. Davies et al.

*Pseudomonas aeruginosa* clinical isolates: these 20 strains were collected from the sputum of CF patients at Carle Foundation Hospital in Urbana, IL, USA. Each strain was subcultured twice on tryptic soy agar (TSA) plates and stored as a glycerol stock at −78°C.

**Preparation of chelated iron sources**

Eight structurally distinct iron chelators (Fig. 1) and FeCl₃ were purchased from Acros Organics (deferiprone) and Aldrich Chemicals (all others) and used without further purification. Stock solutions of chelated iron (50 mmol l⁻¹) were prepared by weighing several milligrams of a given iron chelator into a microcentrifuge tube and adding sufficient 50 mmol l⁻¹ FeCl₃ solution (from MilliQ ultrapure H₂O) so that all ferric ion would be fully chelated by the given chelator. Ratio of chelator : iron was 3 : 1 for all chelators except deferoxamine and diethylenetriamine pentaacetic acid (DTPA), which chelate iron in a 1 : 1 ratio. Solutions were mixed vigorously to homogeneity and filtered through 0.4 μm disposable Millipore membranes (Billerica, MA, USA). Solutions were stored at 4°C or on ice until use and made fresh daily.
Microtitre plate tests

*Pseudomonas aeruginosa* PA14 from an overnight culture was grown in T-broth media (10 g tryptone, 5 g NaCl per litre) to a corrected OD<sub>600</sub> of 0.8 and diluted by a factor of 40 with fresh T-broth media. A 300 μl solution of this diluted bacterial suspension was placed in each well of the first column of a 96-well plate and 150 μl of the suspension was placed in each well of the remaining columns save the final column which was left empty for control wells. Chelated iron sources were added from 50 mmol l<sup>-1</sup> stock solutions to the first column to a final concentration of 50 μmol l<sup>-1</sup>. After mixing, 150 μl of the first column was used to inoculate the second column for a concentration of 250 μmol l<sup>-1</sup> and so on for serial dilutions by a factor of 2. The second to last column was left uninoculated for a concentration of 0 μmol l<sup>-1</sup>. Plates were wrapped in parafilm and incubated in a humidified chamber (to minimize evaporation) at 37°C for 40 h. The plates were gently rinsed under lukewarm tap water to remove planktonic and loosely adherent organisms. After rinsing, the plates were shaken dry and each well of each plate stained with 160 μl of an aqueous 0.1% crystal violet solution in water. After allowing the stain to adhere to the biofilms for 15 min, each plate was again rinsed gently under cool water until no more stain could be rinsed from the plate. Each plate was again shaken dry, inverted and allowed to dry thoroughly for 15 min. Finally, 170 μl of a 30% acetic acid solution was pipetted into each well to desorb the adhered stain back into solution. After allowing 30 min for the adhered stain to dissolve into the destaining solution, the biofilm in each well was quantified via absorbance at OD<sub>590</sub> using a Molecular Devices SpectraMax Plus microtitre plate reader. The same procedure was used for evaluating clinical isolates’ biofilm susceptibility to acetohydroxamic acid and picolinic acid.

Assay for in vitro drug distribution in the human lung

Particle size distribution analysis corresponding to delivery into the human lung was performed using an Andersen cascade impactor. This apparatus uses a calibrated vacuum to pull a nebulized stream of particles through a series of plates, differentiating between large and successively smaller particles as they impact on plates, distributing according to their sizes. An eight-stage Andersen Mark II cascade impactor was utilized along with a SpeedVac High Vacuum Pump that pulled 28.4 l min<sup>-1</sup> of air through the impactor. An 8 ml volume of a 50 mmol l<sup>-1</sup> solution of the chelated iron source to be tested (same as stock solutions) was placed into a Pari LC Plus nebulizer connected to a PulmoAide DeVillbiss compact compressor. The mouthpiece of the nebulizer was then connected directly to the inlet of the cascade impactor. The compressor and vacuum pump were switched on simultaneously and allowed to run for 1–2 min. This duration was chosen by trial and error to allow sufficient chelate to distribute onto the plates for easy analysis, yet not long enough to allow extensive pooling of nebulized solution on any plate. The compressor was then switched off, followed by the vacuum 20 s later to allow the last of the nebulized solution to distribute in the impactor. The impactor was disassembled and the plates were allowed to dry completely. Each plate’s contents were then washed into a separate vial using a
small volume (2 ml) of ultrapure water and re-rinsing with that liquid until no more chelator could be dissolved from the plate’s surface. The volume of all the vials was standardized before reading the absorbance of 100 μl of each at the \( z_{\text{max}} \) for the assayed chelator. The relative amount of chelator on each plate corresponds to the following particle sizes and likely terminus in the human lung according to a scale from the Andersen cascade impactor documentation at http://www.thermo.com/eThermo/CMA/PDFs/Articles/articlesFile_26924.pdf (Table 1).

**Results**

**Chelated iron sources are effective at preventing formation of Pseudomonas aeruginosa PA14 biofilms**

We have reported that simple iron salts, such as ferric ammonium citrate, \( \text{FeCl}_3 \), \( \text{Fe}_2(\text{SO}_4)_3 \), and \( \text{Fe}({\text{SO}})_4 \), can perturb biofilm formation in *P. aeruginosa* (Musk *et al.* 2005). Because diverse iron sources were effective in blocking biofilm formation, we were interested in testing chelated iron sources to see if similar effects would be observed. The reasons for such experimentation are three-fold: first, bacteria are quite adept at scavenging iron from their surrounding environment. It is reasonable to postulate that they would be able to utilize the iron from a subset of the chelated iron forms. Second, ‘free’ iron – mineral iron from salts like \( \text{FeCl}_3 \) or \( \text{Fe}_2(\text{SO}_4)_3 \) – can have pronounced side effects on human epithelial tissues. Large doses of such salts into the human gastrointestinal (GI) tract are known to cause haematemesis (vomiting fresh red blood), diarrhoea and intense abdominal pain (McGuigan 1996). Because the intestinal mucosa is generally amenable to fairly harsh conditions, pulmonary epithelial tissues are likely to be similarly, if not more substantially, affected by the presence of large amounts of free iron. Third, some chelated iron forms are poorly mobilized as iron sources by humans. Two such chelators, deferiprone and deferoxamine, are used as agents to chelate excess iron from humans in the event of acute iron poisoning (Kontoghiorghes *et al.* 2005), and iron chelates of both these agents were included in this study. Thinking in the context of CF therapy, we hoped to identify sources of iron that would be readily assimilated by *P. aeruginosa*, causing biofilm disruption similar to earlier studies while being effectively ‘silent’ to the human body.

To test the effect of various forms of chelated iron on *P. aeruginosa*, we assayed a variety of structurally diverse commercially available iron chelators (Fig. 1) loaded with iron so that no free chelator or free ferric iron would be present in solution. All the iron chelators in this study have incredibly strong affinities for iron (Fig. 1) such that mixing them in correct stoichiometric ratios leaves virtually no free iron in solution. This is an important consideration, given that iron starvation by iron-chelating enzymes has also shown effectiveness in the disruption of *P. aeruginosa* biofilms (Singh *et al.* 2002). Any biofilm disruption would then be a result of the ability of the *P. aeruginosa* to mobilize the iron from that chelated form. Two of the eight chelated iron sources assayed, ferric picolinate and ferric acetohydroxamate, showed similarity to ferric ammonium citrate and other iron salts in their abilities to perturb biofilm formation (Fig. 2).

Similar to previous reports (Musk *et al.* 2005), as iron concentration increased, biofilm increased from 0 to 50 μmol l\(^{-1}\), when the trend reversed and the biofilms of *P. aeruginosa* PA14 were powerfully disrupted, with IC\(_{50}\) ≈ 80 μmol l\(^{-1}\). The other six chelated iron sources did not perturb biofilm formation at concentrations up to 500 μmol l\(^{-1}\).

Because some free (uncomplexed) metal chelators have recently been shown to effect biofilm disruption at moderate concentrations (Banin *et al.* 2006; Junker and Clardy 2007), both picolinic acid and acetohydroxamic acid were tested in their free states in the same biofilm disruption assay. At concentrations up to 100 μmol l\(^{-1}\), these chelators did not affect biofilm formation in PA14. Above these concentrations, only slight bacterial growth inhibition was noted, likely a result of iron starvation. This control experiment confirms that iron, rather than the chelators themselves, is the active inhibitor of biofilm formation.

**Table 1** Andersen cascade impactor particle size distribution and lung destination by stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>Particle diameter ( \mu m )</th>
<th>Likely destination in the human lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.0–10.0</td>
<td>Trachea</td>
</tr>
<tr>
<td>1</td>
<td>5.8–9.0</td>
<td>Trachea</td>
</tr>
<tr>
<td>2</td>
<td>4.7–5.8</td>
<td>Pharynx</td>
</tr>
<tr>
<td>3</td>
<td>3.3–4.7</td>
<td>Primary bronchi</td>
</tr>
<tr>
<td>4</td>
<td>2.1–3.3</td>
<td>Secondary bronchi</td>
</tr>
<tr>
<td>5</td>
<td>1.1–2.1</td>
<td>Terminal bronchi</td>
</tr>
<tr>
<td>6</td>
<td>0.7–1.1</td>
<td>Alveoli</td>
</tr>
<tr>
<td>7</td>
<td>0.4–0.7</td>
<td>Alveoli</td>
</tr>
</tbody>
</table>

**Chelated iron sources distribute effectively in an in vitro model of drug distribution in the human lung**

As a prerequisite for a *P. aeruginosa* anti-biofilm compound being a good therapeutic agent for treating CF lung infections, it would likely need to be readily deliverable to the human lung via nebulization. Thus, all the
Chelated iron sources were assayed in an in vitro model of nebulized drug distribution in the human lung, the Andersen cascade impactor. For optimal delivery to all parts of the lung, nebulized particles of 1–5 μm are generally desired (Dunbar and Mitchell 2005). Larger particles never gain access to the lungs, and smaller particles are readily exhaled before settling out of the airway.

The Andersen cascade impactor is an instrument designed to quantify the sizes of particles delivered by a nebulized stream; it has been utilized extensively to evaluate various drug formulations for potential lung delivery via nebulization (Sermet-Gaudelus et al. 2002; Mitchell and Nagel 2003; Dunbar and Mitchell 2005). The device is a connected series of airtight plates or ‘stages’ with successively smaller holes, or jets, which the nebulized drug is pulled through with a calibrated vacuum source. Each successive plate of the apparatus traps smaller and smaller particles as they achieve sufficient velocity in the jet stream and ‘impact’ on plates according to particle size. At a flow rate of 28.4 l min⁻¹, the particle sizes on each stage are given in Table 1, and correspond directly to those particles’ final destinations upon inhalation into the lung (also summarized in Table 1). Particles impacting plates 3–6 are optimally sized for efficient delivery into the human lung, and the percentage of particles this size in a nebulized sample of drug (%RPF₃₆, recovered particle fraction from plates 3–6) provides a quantitative measure of the ability of a nebulized drug to distribute throughout the lung. Overwhelming percentages of the impacted particles from each of the chelated iron sources were recovered from stages 3–6 of the impactor which correspond to particles between 1 and 5 μmol l⁻¹ in diameter, the ideal particle sizes for distribution throughout the human lung. The percent recovered particle fraction from plates 3–6 (%RPF₃₆) is listed for all chelators in Table 2 and particle distribution plots for ferric acetohydroxamate, ferric picolinate and ferric ammonium citrate are included in Fig. 3. The latter was the most potent anti-biofilm iron source previously assayed (Musk et al. 2005).

As two of the chelated iron sources disrupted biofilm formation and are readily nebulizable into particles amenable for delivery to the human lung, these chelated iron sources were evaluated against clinical isolates of P. aeruginosa collected from CF patients. Distinct clinical isolates of P. aeruginosa from the sputum of 20 CF patients were obtained from Carle Foundation Hospital (Urbana, IL, USA) and tested for dose-dependent response to iron in biofilm production (Fig. 4). Seventeen of these strains produced significant biofilm in the microtitre plate test in 96-well plates. Among those, 15

![Figure 2](https://example.com/figure2.png)  
**Figure 2** Iron chelated by (a) picolinic acid or (b) acetohydroxamic acid effectively prevents biofilm formation by Pseudomonas aeruginosa PA14, similar to (c) ferric ammonium citrate’s effect as was published previously (Musk et al. 2005). Error bars = standard deviation from the mean.

<table>
<thead>
<tr>
<th>Chelate of iron</th>
<th>%RPF₃₆</th>
<th>λₘₐₓ(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,10-Phenanthroline</td>
<td>84</td>
<td>460</td>
</tr>
<tr>
<td>3-Hydroxy-2-methyl-4-pyrole</td>
<td>62</td>
<td>460</td>
</tr>
<tr>
<td>Acetohydroxamic acid</td>
<td>80</td>
<td>460</td>
</tr>
<tr>
<td>Deferiprone</td>
<td>91</td>
<td>460</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>62</td>
<td>390</td>
</tr>
<tr>
<td>Kojic acid</td>
<td>75</td>
<td>390</td>
</tr>
<tr>
<td>Picolinic acid</td>
<td>81</td>
<td>460</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>90</td>
<td>390</td>
</tr>
<tr>
<td>DTPA</td>
<td>NA</td>
<td>(Precipitated)</td>
</tr>
</tbody>
</table>
of 17 (88%) strains exhibited 20% or more reduction in biofilm at 250 μmol L⁻¹ ferric acetohydroxamate while 13 of 17 strains (76%) displayed 50% or greater reduction in biofilm at that concentration. Ferric acetohydroxamate and ferric picolinate were less consistently effective than ferric ammonium citrate (FAC) at inhibiting biofilms in the clinical isolates (the effect of ferric ammonium citrate on these isolates had been shown previously and is reproduced in Fig. 4). However, in 11 of the strains tested (65%), one of the two was more effective than FAC, the most general inhibitor. In 16 of 17 strains (94%), at least one iron source reduced biofilm formation by 50%. This profile of iron sensitivities also suggests that a number of different iron sources might be required in future clinical applications rather than a single generalized iron treatment, depending on the susceptibility of a particular P. aeruginosa strain in a CF patient.

Discussion

The effectiveness of the iron chelates in inhibiting biofilm growth in a majority of these clinically relevant P. aeruginosa strains combined with their favourable distributive properties in an in vitro model of drug delivery to the human lung indicate the potential of iron as a therapeutic agent to treat P. aeruginosa biofilms in the CF airway. Of course, there are obvious concerns with such an approach.

First, a sufficient sputum concentration of iron would need to be reached to cause biofilm inhibition rather than the enhanced biofilm production/adherence apparent in our studies at low exogenous iron concentrations. As P. aeruginosa biofilm formation within the CF lung could feasibly be more or less sensitive to chelated iron than in microtitre plates, it would be ideal to examine such a case utilizing animal models of the CF lung. Unfortunately, the limitations of current animal models for examining P. aeruginosa in relation to its pathogenesis in CF are stark. Myriad studies have helped explain the process by which P. aeruginosa differentiates into a biofilm (Kirisits and Parsek 2006; Musk and Hergenrother 2006). However, attempts to relate Pseudomonas biofilm formation in vitro with virulence in vivo have shown only limited correlation. The lung environment is complex, with multiple native cell types and immune defenses that are not well modelled in current static multi-well assays testing biofilm production. At present, there exists no adequate model for facile evaluation of putative therapeutic strategies for chronic P. aeruginosa infection in the CF patient. While murine models of the CF lung do exist, differences in physiology and scale from human lungs mean that the systems are artificial, failing to produce the spontaneous and chronic bacterial colonization characteristic of the

![Figure 3](image-url)
human infection (Grubb and Boucher 1999; Davidson and M. 2001) With these apparent shortcomings, efforts have been focussed on the cystic fibrosis transmembrane conductance regulator (CFTR)-null ferret’s potential as an in vivo model of human CF. A strong case can be made for optimism in this regard: the ferret’s lung physiology is remarkably similar to that of humans in terms of the level of expression of CFTR and cellular differentiation (Li and Engelhardt 2003). Perhaps in the near future, animal models of P. aeruginosa infection will be sufficiently refined as to allow for facile evaluation of chelated iron’s effects on P. aeruginosa strains grown in a more native lung environ.

Also, the toxicity of chelated forms of the iron to the human lung is unknown and would need to be thoroughly understood to establish a relevant therapeutic index. This is apart from the well-understood systemic toxicity that iron can present; serum iron levels would need to be monitored in patients being treated with iron to prevent systemic toxicity, although the concentrations required are quite high (20–40 mg kg$^{-1}$) (Umbreit 2005) and absorption of inhaled drugs from the lung to the rest of the body is generally poor (Geller et al. 2002).

Among the active iron chelates in this manuscript, acetohydroxamic acid is generally described as toxic to humans as it is an inhibitor of several key enzyme classes, including ureases, cyclo-oxygenases and carbonic anhydrase II. However, this toxicity is manifest only at high concentrations, and acetohydroxamic acid is prescribed under the trade name Lithostat™ as a urease inhibitor to treat patients with struvite kidney stones at high concentrations of 10–15 mg kg$^{-1}$ day$^{-1}$ (Rosenstein and Hamilton-Miller 1984). Intriguingly, iron supplementation is required in many cases (as a co-prescription with Lithostat) in order to circumvent iron deficiency as a result of acetohydroxamic acid treatment. This suggests that iron complexed to acetohydroxamic acid might be effectively sequestered from use by the human body, which would make it an ideal iron delivery agent for high-dose treatment in the CF lung.

Unlike acetohydroxamic acid, picolinic acid is produced by the human body as a natural byproduct of tryptophan catabolism (Bosco et al. 2003). It is nontoxic at high concentrations, and ferric picolinate is a popular form of iron for supplementation to treat various forms of iron deficiency in humans. This would suggest it is readily bioavailable and would thus not be ‘silent’ to the human body like an ideal iron delivery agent. However, no instances of the use of these chelated iron forms in the human lung are recorded in the literature. Bioavailability from this location would likely be drastically lower than from oral dosing, if the precedent from other drugs holds.

Ferric ammonium citrate, too, has been utilized as a well-tolerated iron supplement in humans (Taniguchi et al. 1991) in addition to its current role as a bowel contrast agent in magnetic resonance imaging (Hirohashi et al. 1994). As the ratios of iron, ammonium, and citrate vary from batch to batch of this compound, the extent to which iron is chelated by citrate in this compound likely varies considerably.

In summary, P. aeruginosa uses the biofilm phenotype to effectively establish chronic, incurable infections in patients with CF. Previous research showed that elevated iron concentrations were effective at disrupting biofilms of P. aeruginosa. Because free iron is acutely toxic in humans and thus unsuitable for therapeutic use in CF, nine chelated iron forms were tested for the ability to disrupt biofilm formation in P. aeruginosa PA14 in microtitre plate tests. Iron (III) acetohydroxamate and iron (III) picolinate were both effective in disrupting biofilm formation with moderate potencies in these tests. All the chelated iron forms tested showed superb distributional properties in an in vitro Andersen cascade impactor model for drug distribution in the human lung, suggesting that any of these compounds could be readily delivered directly to the CF lung via nebulization. Both iron (III) acetohydroxamate and iron (III) picolinate were also effective at inhibiting the formation of biofilms in a majority of clinical isolates taken from the sputum of CF...
patients. Taken as a whole, these data serve both to bolster the growing base of literature which shows that elevated iron concentrations cause biofilm perturbation in *P. aeruginosa* and suggest continued examination of chelated iron sources as putative anti-biofilm treatments in the CF lung.

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