Evaluation of the antibiofilm effect of novel gallium-lactoferrin nanocomplex on *Pseudomonas aeruginosa* biofilm

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Adherence of bacteria to implanted medical devices or damaged tissue (wound healing) can become a cause of persistent infections. Bacteria have developed mechanisms by which they can circumvent the effects of antibiotics by forming a slimy layer known as biofilm that can induce subsequent infections and resistance to further antibiotic therapy. Gallium has been previously reported to suppress biofilm formation and growth of bacteria. Moreover, Ga3+ ions can block Fe3+ ion dependent processes because unlike Fe3+, Ga3+ cannot be reduced under the same conditions but sequential oxidation and reduction are essential for many of the biological functions of Fe3+ and crucial energy metabolism of bacteria. Lactoferrin is a ∼80kDa member of the transferrin family of non-heme, iron-binding glycoproteins. It can bind two ferric ions (Fe3+) in two similar, but not identical structural lobe regions. Iron chelating property of lactoferrin is the primary mechanism of its antimicrobial activity. Synergistic action of gallium ions and lactoferrin has been explored in this study for its antibiofilm effect by forming gallium lactoferrin nanocomplex.

Gallium lactoferrin complex (GaLtf) was prepared by conventional reduction complexation procedure from gallium trichloride and lactoferrin. Complex formation was confirmed by DSC and FTIR evaluation. GaLtf was investigated for its ability to inhibit growth of *Pseudomonas aeruginosa* using the disc diffusion method. A constant-depth film fermentor (CDFF; Cardiff University, Cardiff, UK) was employed for the production of *P.aeruginosa* biofilms on hydoxyapatite discs. The growth and control of biofilm at various time intervals (6, 24, 48 and 120 h) were studied in the presence of GaLtf nanocomplex by determining CFU and evaluating the biofilm thickness using Confocal Raman Mcroscope. Tobramycin, 50µg/µl, and ultrapure water were used as positive and negative control respectively.

Agar diffusion assay established the antibacterial action of the GaLtf nanocomposite. The zone of inhibition was found to be 20.5 ± 0.5 mm for Ga-LTf compared with Tobramycin (25± 0.5 mm) and lactoferrin (1± 0.5 mm). This confirmed that the antibacterial action of the Ga-LTf nano complex was mainly due to the presence of Ga3+. Gallium lactoferrin nanocomposite also exhibited significant effect on the viability of *P.aeruginosa* suspension. Antibiofilm studies with CDFF indicated that the inhibition of biofilm growth was comparable with tobramycin at 6 hour (figure 1). Raman chemical mapping also indicated a similar result with significant inhibition of biofilm formation on treatment with GaLtf nanocomplex.

Gallium is a new generation antibacterial ion that has the potential to disrupt iron metabolism in a wide range of bacteria. In this study, Ga-LTf achieved a statistically significant (p<0.05) growth inhibition of *P.aeruginosa* biofilm compared with control, and the short-time exposure effect was comparable with that of tobramycin. The result thus suggests that Ga-LTf may complement currently available antibacterial agents. It is hypothecated that local production of bacterial and neutrophil proteases and the low pH could facilitate Ga3+ ion release from lactoferrin complex leading to high level of Ga3+ ions at the biofilm site preventing or disrupting biofilm formation. The lactoferrin can also scavenge iron form the microenvironment preventing biofilm development and growth. This could be useful for the medical device and wound infections that are resistant to conventional antibiotics treatment.