Abstract

Biofilm is a dense colonization of the microbes encased within the complex extrapolymeric substance secreted by the microorganisms. It is a microbial protective mechanism against the host environmental condition including pH, temperature, macrophages and also to escape the immune response. Biofilm induces the chronic disease condition and persistent inflammation at the site. Moreover, multispecies infection, multidrug resistance and poor antimicrobial response are the common complication associated with biofilm infections. Approximately 60% of chronic infection including diabetic wounds, burn wounds, catheters, artificial joint infection, cystic fibrosis and many other infections are biofilm mediated, which creates the difficult to cure. Basically, the extracellular DNA (eDNA) and alginate (specifically by P. aeruginosa in the cystic fibrosis) present in extracellular polymeric matrix (EPM) of biofilm are the key components responsible for the microbial cell adhesion, aggregation, biofilm proliferation and providing the strength as well as three dimensional structure to the adhered biofilm. The biofilm impedes the penetration and immune response, thereby reduces the antimicrobial susceptibility of the microbes. Therefore, the strategy, having the potential to dismantle the EPM and to facilitate the penetration of the antibiotics is needed. We have used nanoformulation based approaches to overcome the disease specific biofilm in which two different enzymes, DNase-I and alginate lyase have been used to improve the antibiotics susceptibility against biofilm-associated infections by breaking the eDNA and alginate, respectively, present in the EPM.

In our first objective we used the combination of solid lipid nanoparticles (SLNs) along with DNase-I enzyme to target the biofilm infections associated with chronic burn wounds. Silver sulfadiazine (SSD) is widely used in burn wound infections but exhibit

delayed wound healing due to fibroblast and keratinocyte toxicity. While the biofilm induce chronic situation along with low grade persistent inflammation leading to the impaired wound closure. We hypothesized that entrapment of SSD in SLNs will increase the antimicrobial efficacy and reduce the toxicity by enhanced uptake, sustained release and avoiding the direct exposure of the high drug concentration to fibroblast at a time. Moreover, the combination of DNase-I will result in the disruption of biofilm by degrading the eDNA, which constitutes the major component of biofilm and responsible for cell adhesion and strength of the biofilm. Extensive optimization by using Box-Behnken Design (BBD) using Design Expert resulted into the formation of optimized SLNs of desired quality attributes. The process and formulation factors was successfully optimized to get the nanoparticles with desired particles size 295.5±15.4 nm and PDI 0.261±0.023, zeta potential -21.3±1.8 mv and entrapment efficiency 75.9±3.4% possessing the spherical shape and dual drug release pattern. Further, the findings of Fourier transformation infra-red spectroscopy (FTIR), differential scanning calorimetry and x-ray diffraction inferred that SSD had no physicochemical interaction with other components and was entrapped efficaciously in its amorphous state. At the same time, the results of MTT assay showed the improved cell viability due to SSD-SLNs (90.3±3.8%) as compared to SSD alone (76.9±4.2 %). Most importantly, the SSD-SLNs with DNase-I inhibited around 96.8% of biofilm, whereas, the SSD with DNase-I inhibited only 82.9% biofilm of *Pseudomonas aeruginosa*. Eventually, the results of *in-vivo* wound healing study showed complete wound healing on day 21 in case of developed SSD-SLN with DNase-I.

In the second strategy, we targeted the alginate abundant biofilm of P. aeruginosa in cystic fibrosis using the alginate lyase in combination with ciprofloxacin loaded chitosan nanoparticles. Basically, dense colonization of mucoid *Pseudomonas*

aeruginosa within the self-secreted extracellular matrix (mainly alginate), called biofilm, is a principal reason for the failure of antimicrobial therapy in cystic fibrotic patients. Alginate is a key component in the biofilm of mucoid *P. aeruginosa* and responsible for the surface adhesion and stabilization of biofilm. To overcome this problem, alginate lyase functionalized chitosan nanoparticles of ciprofloxacin were developed for the effective treatment of *P. aeruginosa* infection in cystic fibrosis patients. The developed nanoparticles were found to have desired quality attributes such as 205.5±9.0nm PS, 0.302±0.031 PDI, 12.2±2.1mV ZP and 51.8±2.1% EE and demonstrated spherical shape and sustained release following the Higuchi release kinetics. Drug compatibility with the chitosan was confirmed by FTIR while powder X-ray diffraction analysis confirmed the entrapment of homogeneous drug dispersion in its molecular state within the nanoparticle matrix. Lactose adsorbed NPs showed promising aerodynamic property. Nanoparticles showed prolonged MIC and significant reduction in biofilm aggregation and formation in planktonic bacterial suspension. Nanoparticles exhibited significantly higher inhibitory effect against biofilm of *P. aeruginosa* and reduced the biomass, thickness and density as confirmed by confocal microscopy. Furthermore, developed nanoparticles were hemocompatible and did not exhibit any toxicity *in vitro* and *in vivo*.

Thus, data in hand collectively suggests that DNase-I along with nanoparticles can be an effective approach to treat the biofilm-associated infections having eDNA as key component. However, the alginate rich biofilm, specifically associated with P. aeruginosa infection in CF patients, can effectively be eliminated using the alginate lyase as biofilm dispersing agents along with nanoformulations.