Abstract

Microbial biofilms are surface attached colonies of microbes surrounded by self-produced extracellular matrix. These biofilm cause chronic infections which results in increased cost of treatment and prolonged hospitalization time. Biofilm architecture provides bacteria with enhanced antibiotic resistance, thus raising the need to search for alternative therapies that can inhibit the bacterial colonization. Nanotechnology based approaches are being employed for development of nanoparticles and nanocomposites which may be used to circumvent biofilm associated infections. The aim of our study was to synthesize and characterize different nanomaterials and to investigate their applicability in reduction of bacterial biofilms.

We initiated this study with the formation of graphene/zinc oxide nanocomposite (GZNC). The synthesized GZNC was characterized by UV-visible absorption spectroscopy, X-ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FTIR), Thermo gravimetric analysis (TGA) and Transmission electron microscopy (TEM). The results revealed the formation of well dispersed zinc oxide nanoparticle onto the surface of graphene oxide nanosheets. Further, the prospective of GZNC against the cariogenic properties of Streptococcus mutans like adherence, exopolysaccharide formation, acid production, acid tolerance and obstinate biofilm formation was explored. The anti-biofilm behaviour of artificial acrylic tooth surfaces coated with GZNC was also examined. Acrylic teeth are good choice for implants as they are of low cost, have low density and can resist fracture. Microscopic studies and anti-biofilm assays illustrated a significant reduction in biofilm in the presence GZNC. It was also found to be nontoxic against HEK-293 (human embryonic kidney cell line). The results indicate the potential of GZNC as an effective coating agent for dental implants by efficiently inhibiting S. mutans biofilm.

In second study, sub inhibitory concentrations of calcium fluoride nanoparticles (CaF$_2$-NPs) were assessed for their effect on biofilm forming ability of S. mutans in vivo and in vitro models. CaF$_2$-NPs were characterized using various techniques (TEM, XRD, FTIR and UV-visible spectroscopy). The in vitro studies revealed 89% and 90% reduction in biofilm formation and EPS production respectively. Moreover, acid production and acid tolerance abilities of S. mutans were also reduced considerably in the presence of CaF$_2$-NPs. Confocal laser microscopy and transmission electron microscopy images were in
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accordance with other results indicating inhibition of biofilm without affecting bacterial viability. The qRT-PCR gene expression analysis showed significant down regulation of various virulence genes (vicR, gtfC, ftf, spaP, comDE) associated with biofilm formation. Furthermore, CaF\textsubscript{2}-NPs were found to substantially decrease the caries in treated rat groups as compared to untreated in \textit{in vivo} studies. Scanning electron micrographs of rat’s teeth further validated our results. These findings suggest that CaF\textsubscript{2}-NPs can be used as an antibiofilm agent against \textit{S. mutans} and may be applied as a topical agent to reduce dental caries.

In our third study, we have reported a non-toxic and eco-friendly route for synthesis of graphene oxide-silver nanocomposite (GO-Ag) using a floral extract of \textit{Lagerstroemia speciosa} (L.) Pers. plant. Nanocomposite was characterized using TEM, UV-visible spectroscopy, XRD and EDX (Energy-dispersive X-ray spectroscopy). Sub inhibitory concentrations of green synthesized GO-Ag reduced the biofilm formation in both gram-negative (\textit{Enterobacter cloacae}) and gram-positive (\textit{Streptococcus mutans}) bacterial models. Growth curve assay, membrane integrity assay, scanning electron microscopy (SEM) and confocal scanning laser microscopy (CSLM) revealed different mechanisms of biofilm inhibition in \textit{E. cloacae} and \textit{S. mutans}. Biofilm inhibition in \textit{E. cloacae} was due loss of viability of planktonic cells while in \textit{S. mutans} there was no loss of viability. Moreover, quantitative RT-PCR (qRT-PCR) showed significant down regulation of \textit{vicR}, \textit{spaP} and \textit{comDE} genes which play crucial role in \textit{S. mutans} biofilm formation, suggesting GO-Ag is acting on its biofilm formation cascade. Antibiofilm concentrations GO-Ag was also found to be non-toxic against HEK-293. The whole study highlights the therapeutic potential of GO-Ag to restrain the onset of biofilm formation of both gram-negative and gram-positive bacteria although its mode of action is species specific.