ABSTRACT

Bacterial contamination aided by antibiotic resistant biofilm formation on abiotic surfaces is a major concern in healthcare and industrial environment. Antibacterial coatings on such surfaces are considered an effective way towards addressing these issues. A number of suitable antibacterial agents, including antibiotics, metal nanoparticles, quaternary ammonium compounds (QACs), antimicrobial polymers, have been investigated for such antibacterial surface coatings. However, emergence of multidrug resistant bacteria has emphasized the need for an effective alternative. A potential substitute is antimicrobial peptides (AMPs), class of short polypeptides usually associated with the host organisms innate immune system. AMPs exhibit broad spectrum antimicrobial activity, low propensity towards pathogen resistance, and low immune response. These properties make AMPs an ideal candidate to be used in antibacterial coatings in preventing bacterial adhesion and/or survival on commonly used surfaces. However, maintenance of peptide activity and stability after immobilization is a crucial prerequisite for the development of AMP-based surface coatings.

A short (12 residues), random-coiled, pore-forming antimicrobial peptide (AMP) named KLR (KLLLRLRKLLRR), is designed based on a proposed 'design rule' that optimizes the cationicity and hydrophobicity of peptide. KLR is immobilized on multiple surfaces, namely polystyrene (PS), stainless steel (SS), and glass, to investigate the effect of surface immobilization chemistry on antibacterial efficacy of the designed AMP. Since PS, SS, and glass have wide applications in medical sector, therefore, bestowing them with antibacterial properties would be of great advantage. The effect of peptide orientation on its antibacterial activity is further studied by adding a cysteine residue at N-terminal of peptide, named CKLR (CKLLLRLRKLLRR), followed by its immobilization. The surfaces were analyzed by atomic force microscopy (AFM), water contact angle (WCA) measurements, and X-ray photoelectron spectroscopy (XPS), to confirm chemical modifications at each step. The findings show that KLR

immobilized on to PS and SS surfaces results in almost complete inhibition of both *E. coli* and *S. aureus* irrespective of the orientation. The peptide-coated glass surfaces, however, inhibited *E. coli* but were ineffective against *S. aureus*. All peptide-modified surfaces were non-cytotoxic towards fibroblasts. Surface segregation of peptides determined from bromophenol blue (BPB) binding method indicated 20-25% of the total available peptide binds to PS surface. Microstructural examination, micro-hardness, and tensile test on peptide modified stainless steel surfaces (SS-KLR) suggest mechanical properties of SS-KLR substrates are unaffected by the chemical treatment employed for surface modification. Immobilized KLR on glass surfaces show pore-forming mechanism of action, thereby, suggesting less likeliness of development of antibiotic resistance. Thus, versatility of KLR makes it a potential antibacterial candidate for development of broad-spectrum antibacterial coating on multiple biomaterial surfaces.