Studies on the Preparation of Antimicrobial Polyurethane for Biomedical Application

ABSTRACT

In the advanced era of biomedical engineering the implants reside within human bodies either temporarily or permanently for restoring body functionality, diagnostic, monitoring, and therapeutic purposes. The choice of implant material (natural or synthetic polymer) and duration of implantation depend on the final application. Polyurethane (PU) has gained prominence in the field of biomedical devices and implants due to its tailor-made properties, biocompatibility, hemocompatibility, and ability to mimic natural tissues. The challenging part with PU implants is their chemical inertness, thrombosis formation, and the lack of cell adhesive ligands. Chemical functionalization techniques can introduce different functional groups on the PU surface. The generated functionality helps to immobilize bioactive components which reduces the occurrence of thrombosis and enhances the interface-cell interaction of PU implants.

Through the use of hydrolysis and aminolysis, the PU surface has been chemically functionalized. Alkaline hydrolysis using sodium hydroxide (NaOH) was used for the generation of hydrophilic (amine and hydroxyl) functionality on polyurethane surface. The surface morphology of hydrolyzed polyurethane was characterized using SEM and AFM technique, which confirmed the formation microdomains on the PU surface due to hydrolysis. These microdomains altered the transparency of the films and made the film opaque in appearance. PU film was subjected to aminolysis using ethylene diamine (EDA) under varied temperatures, time, and EDA concentrations. The influence of reaction parameters on the formation of free amino ($-NH_2$) groups and the resulting changes in the physicochemical properties of PU were investigated further. Aminolysis process was selected over hydrolysis because similar amine content was achieved in less time and at a lower temperature with relatively less weight loss and tensile strength loss.

Carboxymethyl Cellulose (CMC) is utilized as a coating material due to its superior water absorption, nontoxicity, pH sensitivity, biodegradability, and biocompatibility. Following the modification of the polyurethane surface *via* aminolysis, the CMC polymer is covalently immobilized via Schiff base chemistry. The periodic oxidation of CMC results in the formation of dialdehyde groups along the backbone of the CMC chain. An imine bond is created when these aldehyde groups OCMC interact with the amino groups on aminolyzed PU (PU-A) surface. Scanning electron microscopy (SEM), contact angle, and X-ray photoelectron spectroscopy (XPS) measurements are used to characterize and validate the immobilization of OCMC on aminolyzed PU film (PU-O).

Nitrofurantoin (NF) drug is used to incorporate within the OCMC gel and to immobilize on the PU surface for creating antimicrobial PU surface. The incorporation of NF in OCMC resulted in a moderate increase in WCA. The cross-sectional morphology of PU-ON films was investigated using FESEM. The incorporation of the drug is confirmed using EDX analysis. NF has shown the concentration dependent bacteriostatic and bactericidal activity against Gram-positive and Gram-negative bacteria. The PU-ON showed a drastic reduction in bacterial

adherence. The bacteria were vastly inflated and had ruptured the bacterial cell membrane, confirming that the adhered bacteria were dead.

The designing of nanosilver (nAg) has been carried out by *in-situ* reduction of silver nitrate using oxidized carboxymethyl cellulose (OCMC). The reduction process was also accompanied by the stabilization of nAg particles using the OCMC polymer chain, leading to the formation of a structure where nAg was entrapped within OCMC gel. The nAg particles have exhibited prominent antimicrobial activity against both gram-negative and gram-positive bacteria. The antibactericidal effect of the nAg particles was further investigated by TEM and reactive oxygen species (ROS) generation after nAg particle treatment. It showed strong intracellular fluorescence and also showed that OCMC-nAg particles produced intracellular ROS in *E. coli* and resulted in cell death.

There is a good possibility to use a blend of nAg particles and drug to address the concerns with cytotoxicity of nanoparticles and resistance to antibiotics. The synergistic effect of NF and nAg particles was assessed through *in-vitro* experiments. The NF and nAg particles were blended in different blend solutions, such as 100:0, 90:10, 80:20, 70:30 and 60:40. There was a layered structure with nAg particles distributed in the top layer. Further in EDX analysis, the emergence of peak at ~2.9 KeV, which is the characteristic peak of Ag, confirmed the presence of nAg particles in the coating. The NF and nAg particles solemnly were not capable of giving the prolong antimicrobial activity, but the blends showed prolonged activity due to synergistic effects. The 70:30 blend ratio was carried forward for further study.

Urinary catheterization in hospitals leads to nosocomial infections and high mortality rate. The major cause of nosocomial infection is the development of encrustation and bacterial biofilm on the surface of the urinary catheter. The infection resistance coating having (70:30) NF:nAg particles, was utilized to design antimicrobial catheter. Later, OCMC-NF/nAg blend was immobilized on the functionalized catheter, exploiting Schiff base chemistry. The bacterial colony formation was nearly completely suppressed by the PU-ON/nAg (70:30) catheter, whereas many colonies could still be found with PU catheter. PU-ON/nAg (70:30) catheter was found to be resistant to the bacterial colonization, and no trace of bacteria was detected. Encrustation is a significant urinary catheter problem in addition to bacterial adhesion. Encrustation, *i.e.*, deposition of salt on the surface, was gradually developed with time in the urinary environment. After flowing synthetic urine for 7 days, encrustation was examined using SEM. The number of encrustations on the PU-ON and PU-ON/nAg catheter significantly decreased. The antibacterial migration assay was carried out through an *in-vitro* model. In PU catheter bacterial migration was prominent, as a dense biofilm was formed on the section in the vicinity of a bacterial suspension. These results indicated that both catheters may not be able to impede bacterial adhesion and migration in a nutrient-rich culture medium. In comparison with the above, the PU-ON and PU-ON/nAg catheters effectively inhibited bacterial migration. The histopathological evaluation of catheter in Swiss albino mice showed that the catheter is biocompatible in matrix. The present investigations therefore lead to the insight of the PU catheter development which is antimicrobial and biocompatible in nature.