8.1. Introduction

The presence of a foreign body implant enables skin derived bacteria to become pathogenic. After adhesion to the implant surface, CNS produce an extracellular mucoid biofilm that embeds the growing cells. This is acting as a barrier that prevents penetration of antibiotics. The key to effective prophylaxis of infection seems to be the prevention of initial bacterial adhesion by antimicrobial device impregnation or coating. In the present study efficacy of a technique of incorporating an antibiotic into a silicon polymer in preventing surface colonization has been evaluated by the use of scanning electron microscopy (SEM) and culture methods. The study was conducted using rifampicin which was found to be effective by the antibiotic sensitivity test done previously under chapter-5. The most predominant CNS species *S. epidermidis* was selected for the study.

8.2. Materials and Methods

Silicon catheters for urinary catheterization (Ramson Scientific and surgical industries Pvt. Ltd. Agra) were cut into 1cm pieces. Rifampin was chosen because of its high physicochemical compatibility with the silicon matrix and its good activity against staphylococci. Rifampin was added to silicon catheters (in chloroform) until a concentration of 9% was reached.
Removal of the solvent chloroform by controlled evaporation under sterile condition was achieved (1).

An overnight culture of *S. epidermidis* strain (strain HCS-102) which was positive for slime and biofilm and sensitivity to rifampin was chosen for this study. The culture was diluted with nutrient broth. 300ml volumes of this bacterial suspension were added to each 500ml conical flask for bacterial colonization of catheters in vitro. A control with no added bacterial suspension was also included. These conical flasks were sealed with a plastic plug in which the 1cm rifampicin impregnated silicon catheters were pinned to needle shaped tips which is inserted through the plastic plug. Aeration was achieved through an additional filter covered plug hole. The whole system was placed in a water bath and incubated at 37°C (Plate 17).

**Plate 17:** The experimental setup to study the effect of rifampicin impregnation of silicon catheter on biofilm formation
Two catheter pieces were removed from each test system after 30Min, 3h, 12h and 24h respectively; one for culture and other for SEM study. To remove non-attached bacteria, the catheter was gently rinsed with 20ml of 0.9% NaCl solution and was washed in running water for 2 min. Then it was shaken to remove excess fluid and was rolled over a blood agar plate, according to the method of Maki et al.,. The plate was incubated for 18h at 36°C, and the resultant colonies were visually counted to detect the presence of viable bacteria adherent to the foreign body surface. For SEM study the catheters obtained from the in vitro experiments were fixed with 2.5% gluteraldehyde in 0.1 m sodium phosphate buffer (pH 7.3) for 24h and the scanning electron microscopy (SEM) was performed.

8.3. Results

The SEM examination of the surface of an unused silicon catheter surface showed a very rough appearance (Plate: 18). The surface irregularities of this unused catheter act as sites for bacterial adherence. The rifampin impregnated silicon catheters were covered with rifampicin crystals of different sizes and shapes. This surface appears to be even rougher than the original catheter. The surface of the catheters removed from the bacterial suspension after 30min (Plate: 19) and 3hrs (Plate: 20) showed no sign of bacterial colonization. But after 12 hrs exposures very few bacterial cells were seen on the surface of the catheter (Plate: 21) without any sign of slime production. At 24 hrs after bacterial exposure, the surface of the catheter was covered by colonies of bacteria with slime matrices (Plate: 22). Because of the presence of rifampicin on the catheter surface, no growth was observed in blood agar plates of 30 min and 3 hrs exposures. But after 12 hrs exposure plate, very few colonies of bacteria were found. At 24 hrs, S. epidermidis
colonies were recovered in large numbers and were positive for slime production.

**Plate18:** SEM of surface of an unused silicon catheter

**Plate19:** SEM of rifampicin impregnated catheter after 30min
Plate 20: SEM of rifampicin impregnated catheter after 3 hrs

Plate 21: SEM of rifampicin impregnated catheter after 12 hrs
8.4. Discussion

The colonization of catheters with bacteria is influenced by different factors. Locci et al., (1981) have observed intrinsic irregularities on the surface of unused catheters by SEM. Amorphous deposited substances or glycocalyx have been noted surrounding bacterial colonies on the surface of catheters in vitro (Christensen et al., 1982). Studies on this suggest that the deposited substances are exopolysaccharides which can be produced by CNS (Christensen et al., 1985). This biofilm seems to protect the bacteria from host defense mechanisms by specific and non-specific interference with the immune system. In addition, it acts as a barrier to antibiotics (Anwar et al., 1992). Different studies were carried out to take a possible preventive approach by incorporation of antibiotics on to or in to the catheters providing initial and long lasting bactericidal antibiotic concentrations at their surface (Christenson et al., 1983). Bayston et al., (1989) described a new
impregnation procedure capable of conferring antimicrobial protection against CNS for up to 28 days. The present study evaluated the in vitro efficacy of rifampicin impregnated catheter in preventing the surface colonization of \textit{S. epidermidis} by SEM and culture methods.

Examination of catheter was done after different exposure time, 30min, 3h, 12h and 24h of this, no visible bacterial colonization were observed after 30min and 3hrs of exposure. No sign of colony formation or slime production were visualized. Results obtained in culture studies also confirmed this observation. This indicates the bactericidal effect of rifampicin impregnated on the polymer surface. Recent efforts aimed at reducing the incidence of biomedical implant infections have relied upon improvements in aseptic handling techniques and site care (Segura et al., 1989), reduction of bacterial pathogen reservoirs by using prophylactic antimicrobial agents (McCullough et al., 1980), modifications in catheter design and the use of materials that decrease the likelihood of bacterial contamination (Trooskin et al., 1985). Future strategies may include the development of agents or antimicrobial coatings which inhibit or suppress the primary adherence of \textit{S. epidermidis} to the surface of biomedical implants, thus circumventing the production of a biofilm layer by slime-producing strains. In the present study after 12 hrs exposures very few \textit{S. epidermidis} cells were seen on the surface of the catheter without any sign of slime production. Kockro et al., (2000) have reported the results of antibacterial effect of rifampin impregnated catheters, which differ from the observation in the present study. Colonies were developed after 3 hrs of exposure in their study. This may be indicating strain to strain variation in extent of slime production or sensitivity to rifampicin. In the study though blood agar cultures of catheters showed no growth after 30 min and 3 hrs
exposures, a large number of \textit{S. epidermidis} strains with slime production were observed after 24 hrs. It may also be possible that adherence to the surfaces of foreign objects may allow organisms to become metabolically dormant; thus, they may persist on foreign body materials in an occult fashion until a clinical infection develops. Slime productions contribute to the pathogenicity of CNS in association with prosthetic devices. However, after initial adherence, the presence of various amounts of slime enveloping catheter-adherent bacteria is likely to form an impervious barrier against in vivo nutrients, further allowing organisms to persist in growth limiting environment, a status comparable to our in vitro experimental system. The slime producing isolates produced significantly higher densities of organisms in the catheter. These data support the ultrastructural scanning electron microscope observations of Peter \textit{et al.}, (1982). These authors found no evidence of slime production during the initial adherence to catheters, but, with progressive colonization, slime was increasingly noted and there was a concomitant increase in the number of staphylococcal cells adhering to the catheter. Isolates used in the present study were exhibiting variations of differences from the isolates used in studies conducted abroad, with respect to their phenotypic properties. Taking this into accounts the present study was conducted on the effect of rifampicin on biofilm formation, assuming that there can be differences in sensitivity to rifampicin among strains from different geographical areas. In the present study bacterial exposure after 24 hrs, the surface of the rifampicin impregnated catheter was covered by \textit{S. epidermidis} with slime matrices. The pathogenesis of biomedical implant infections caused by \textit{S. epidermidis} can be separated into at least two distinct phases (i) primary bacterial adherence to the surface of the device and (ii) the production (by certain strains) of a stable biofilm and matrix over the surface
of the device. Primary adherence is initiated through hydrophobic or ligand-specific interactions between the exterior surface of the cell wall and the surface of the device (Hogt et al., 1986). Biofilm production is an attribute of strains of *S. epidermidis* which elaborate an extracellular polysaccharide or slime (Christensen et al., 1983). In vivo, the slime polysaccharide complex with host factors to form an extracellular matrix which anchors bacteria firmly to the surfaces of biomedical implants (Peters et al., 1981) and protects adherent organisms from defenses (Gray et al., 1984). Sterilization of a biomedical implant infected by *S. epidermidis* without surgical revision or removal is difficult, especially if the offending strain produces a slime polysaccharide (Dunne WM Jr., 1990).

Rifampicin has been noted to exhibit exceptional antimicrobial activity against *S. epidermidis* biofilms, as compared to commonly used antibiotics. It penetrates biofilm adequately and also reduces the adherence of bacteria to surfaces. In clinical practice rifampicin addition has been occasionally demonstrated as beneficial in terms of clinical or bacteriological cure rates especially for prosthetic device related infections. In contrast to the specimens obtained at 30 min and 3 h, SEM and growth in blood agar plates of the rifampicin-impregnated catheter removed at 12 h and 24 h showed an increased number of bacteria surrounded by a typical biofilm. This confirms the differences in antibiotic sensitivities exhibited by strains from different geographical areas. These observations clearly demonstrate that prevention of bacterial colonization was dependent on the bactericidal effect of the incorporated rifampicin and show the necessity for higher antibiotic loading. Rifampicin is well known for the development of single-step resistance (Kadurugamuwa et al., 2004), hence a combination with another antibiotic may be adequate to exclude the possibility of the
development of colonization. Further studies are required to investigate the feasibility of incorporation of a combination of rifampicin with other antibiotics in to silicone polymer to minimize the risk of development of resistance against rifampicin.