5. SUMMARY OF THE REPORT

Human tissues often support large, complex microbial communities growing as biofilms that can cause a variety of infections. Due to an increased use of implanted medical devices, the incidences of these biofilm-associated diseases are increasing: the non-shedding surfaces of these devices provide ideal substrata for colonization by biofilm-forming microbes. The consequences of this mode of growth are far-reaching. As microbes in biofilms exhibit increased tolerance towards antimicrobial agents and decreased susceptibility to host defence systems, biofilm-associated diseases are becoming increasingly difficult to treat. Hence in the present research, fabrication of biomedical products with synergistic antibacterial drugs and carriers were extensively studied to prevent such drug tolerant capacity of microbes in biofilms.

Since the molecular and physical interactions that govern bacterial adhesion to biomaterials have not been understood in detail all the available preventive measures that decrease the rate of bacterial infections should be taken (Katsikogianni and Missirlis, 2004). The preventive strategies described by these researchers were: experienced therapy teams to insert and maintain indwelling devices, maximum sterile barriers, such as sterile gloves, masks, gowns, caps, large drapes and careful handwashing. Use of these precautions has been linked to a four-fold decrease in the rate of bacteriaemia. Moreover, cutaneous antimicrobials and antiseptics, ionic silver cuffs, combination of antibiotics with heparin, antiseptic hubs and antimicrobial coatings of biomaterial surfaces have shown good results against microbial colonization and produced bacteriaemia, especially when the right antibiotics are chosen against each type of bacteria. Based on these significant factors, in the present research antimicrobial coatings on the materials to provide ultrasmooth surface for preventing the bacterial adhesion and biofilm deposition were extensively studied. To meet this objective the materials were surface coated with three different polymer based drug-carriers grafted with the right set of synergistic antibacterial agents. Two significant objectives were fulfilled by the method of dip-coating the test materials. One
among them was coating the suitable drug-carrier for sustained release of drugs which eventually meant that the rate of degradation of the carrier was proportional to the rate of drug release. This was proved during the in vitro dissolution studies and in vitro challenge test carried out in the present research. Second one being the mode of action of synergistic drugs. Fluoroquinolone and nitroimidazole drugs used to coat the materials were of similar action on targeting the DNA and its significant enzymes both in vitro and in vivo. So that gaining resistance by the biofilm producers against the usual oral antibiotics was prevented.

*Overall summary of the present research was presented below*

MIC of each fluoroquinolone and nitroimidazole drug was observed to be more than the MIC of drugs in combinations. The FIC concentrations of ofloxacin and ornidazole indicated the synergism against aerobic and anaerobic organisms. The synergistic drug concentrations of all three drug combinations in the present study showed good antibacterial activity when tested qualitatively. Drug release analysis showed that the concentrations of each drug were more than the MIC and less than the pathogenically relevant dose. Since the concentration was more than MIC it would be essential at the implanted site and its surrounding tissues for the prevention of bacterial adherence. Bacterial adherence study was analysed to prove this factor. In the study it was proved that the released drug concentrations from the materials prevented the production of bacterial adhesins produced by the test bacteria from the number of CFU obtained for each test bacteria against the coated materials. *In Vitro Challenge (IVC) test* proved the duration and persistence of antibacterial activity of drugs coated on the materials. The IVC test results were also influenced by the drug concentrations released from the materials. Qualitative tests, bacterial adherence, and IVC test results showed the different characteristic features of the drug-carriers used in the study. In all these parameters, the carriers facilitate the antibacterial activity of the synergistic drugs to a significant level. The influence of drug concentrations in the qualitative test and IVC test proved that the carriers release the drugs at a sustained level, so that the
concentration of drugs released at regular intervals were significant in preventing the biofilm formation. From the tested parameters, the result showed that at least one drug-carrier combinations was proved to be more effective for each implantable material.

**Implantable Materials**

For silicone, Ofloxacin-Ornidazole + DL-lactic acid (D_2C_2) proved to be more effective against both aerobic and anaerobic organism. Bayston et al., (2009) reported that antibiotic coated CSF silicone shunts prevented biofilm forming organisms associated with CSF hydrocephalus shunt infections.

PTFE stents are associated with coronary artery diseases caused by *S. epidermidis*. In the present research the PTFE samples coated with norfloxacin-metronidazole + DL-lactic acid (D_1C_2) were proved to be effective against the test bacteria *S. epidermidis* (ATCC 35984) and *S. aureus* (ATCC 29213). Poly DL-Lactic Acid (pDLLA) as drug carrier was used by Matl et al., (2008) to coat PTFE grafts with gentamicin and teicoplanin to retard the surface colonization of *S. epidermidis*. Similar type of carrier was used in the present research facilitates the antibacterial activity, sustained drug release profiles and biocompatibility.

Polyurethane catheters influences urinary tract infection and intravascular catheter related infections. It was mainly caused by many Gram-Positive and Gram-Negative bacteria. Different antibacterial coated urinary and intravascular catheters made of polyurethane were already proved to be more antibacterial according to El Rehewy et al., (2009) and Yorganci et al., (2002). In the present study, polyurethane coated with Ofloxacin-Ornidazole + β-Cyclodextrin (D_3C_1) was proved to be more effective against both aerobic and anaerobic organisms. Brewster and Loftsson, (2007) reported that β-Cyclodextrin cyclodextrins (CDs) have been found as potential candidates in drug delivery systems since they have the ability to alter physical, chemical and biological properties of guest molecules through the formation of inclusion complexes. It has been widely used in pharmaceuticals due to these applications.
Silk sutures are highly associated with surgical-site infections due to *S. epidermidis* and *S. aureus*. In the present study silk sutures coated with Ofloxacin-Ornidazole + Tocopherol acetate (D2C3) were proved to be significantly more antibacterial. Fryer, (1993) reported the application of Tocopherol acetate which plays an important prophylactic role against several serious light induced diseases and conditions of the eye and skin that are mediated by photo-oxidative damage to cell membranes. In addition to the cited literature, Tocopherol acetate also provides a sustainable release of drugs according to Matl et al., (2008) during the *in vitro* drug release analysis aiming that it has direct influence on inhibiting bacterial adherence on the material surface.

Qualitative antibacterial activity was carried out for all the drug-carrier coated materials against the test organisms based on the synergistic activity after checker board analysis. For each material different drug-carrier combinations were tested against all the test organisms. Based on the results of qualitative analysis three effective drug-carrier combinations were selected for drug release analysis (silicone- D1C1, D2C2, D3C2, PTFE-D1C2, D2C3, D3C2, Polyurethane- D1C1, D2C1, D3C1, polyester- D1C1, D2C1 D3C2 and silk suture- D1C3, D2C3, D3C1). [Where, D1, the combination of norfloxacin and metronidazole; D2, combination of ofloxacin and ornidazole; D3, combination of ciprofloxacin and tinidazole; C1 - beta-cyclodextrin; C2 - DL-lactic acid C3 - tocopherol acetate]. The idea was to find out at least one effective drug-carrier combination for each implantable material.

Drug release studies showed that the drug-carriers, β-Cyclodextrin, DL-lactic acid and tocopherol acetate were involved in providing a slow and sustained release of drugs from the material surface. This was confirmed by comparing the drug release profiles of *drug-carrier coated* (dcc) implantable materials and *drug-coated* (dc) implantable materials (drug without carrier). After an initial burst release concentration of drugs there was a steady and constant release of drugs were observed from the *drug-carrier coated* (dcc) implantable materials whereas after the initial burst period the release concentration was not constant in the case of drug alone coated (dc) materials. Based on the results of
drug release analysis one effective drug-carrier providing constant and sustained release of drugs were selected for each type of material (silicone-D_{2}C_{2}, PTFE-D_{1}C_{2}, Polyurethane- D_{2}C_{1}, polyester-D_{2}C_{1} and silk suture-D_{2}C_{3})

For each material coated with selected drug-carrier combinations bacterial adherence test was carried out to identify the ability of bacteria to adhere on the coated material surfaces. All the test bacteria showed sensitivity against the selected drug-carrier combinations. The bacterial reduction percentage of drug-carrier coated and carrier coated materials was statistically calculated using chi square test. The differences in bacterial adherence between the drug-carrier coated and carrier coated were statistically calculated considering P<0.05 significant. The drug-carrier coated implantable materials showed more bacterial reduction percentage (P<0.05) than the carrier coated implantable materials (P>0.05). In Vitro challenge test was carried out to analyse the persistence and duration of antibacterial activity of drugs on the material surfaces. The IVC test report suggests that the drug-carrier coated materials restricted the growth or surface colonization of test bacteria till 5 challenge doses (30 - 35 days). The antibacterial duration of 30 to 35 days could be reliable and sufficient for any patient so that the initial chance of getting the implants contaminated would be eliminated. This can decrease the rate and numbers of susceptible persons from the dreadful implant-associated infections (IAI), biomedical product associated infections (BMPAI) and surgical-site infections (SSI).

**Non-implantable Materials**

Permanent or durable binding of inorganic compounds to organic substrates is extremely difficult, especially for mass production processes. By utilizing the properties of synergistic drugs, an inexpensive platform technology was developed which may permanently binds it to textile fibres by which woven and non-woven fabrics can be fabricated. The introduction of synergistic drugs at the early stages of the textile production cycle enables the use of cotton and other fibres in many manufacturing processes without altering manufacturing
procedures or equipment, allowing for rapid and simple production of fabrics with potent biocidal qualities. The possibility of introducing the synergistic drugs into fabrics may have significant ramifications. One example is the reduction of nosocomial infections in hospitals. The main sources for contamination are the patient’s skin flora, the flora on the hands of medical and nursing staff, and contaminated infusion fluids. However, recently it has been demonstrated that sheets which are in direct contact with a patient’s skin and his bacterial flora are an important source of infection. Moreover, sheets are significantly more contaminated by patients carrying infection than by non-infected patients. Therefore, use of fabrics that kill microbes in sheets, pillow covers, and robes in a hospital setting, may reduce the spread of microorganisms in hospital wards, resulting in a reduction of nosocomial infections. Thus, the use of fabrics with biocidal properties in a hospital setting may not only reduce hospital mortality and morbidity, but may also significantly reduce hospital and insurance costs.

Based on all these factors, in the present research for each textile material, atleast one drug-carrier combination was found to be effective for antibacterial finishing methodologies. From the reactive dye method it was analysed that the drugs could withstand only upto 10 washes whereas, the microencapsulated drug treated textile materials showed resistance till 15\textsuperscript{th} wash.

In Reactive dye method the textile materials were treated with three different synergistic drug combinations along with three different drug-carriers. The textile materials treated by Reactive dye method showed good antibacterial activity and percentage reduction against all the test bacteria. The drug-carriers grafted with the synergistic drugs provide good resistance against wash fastness. The treated reactive drugs withstand till 10 washes in all the textile materials. After analysing all the textile materials treated with reactive drugs and carriers, atleast one effective drug-carrier combination was selected. D\textsubscript{2}C\textsubscript{1} was found to be the effective drug-carrier combination for cotton; D\textsubscript{2}C\textsubscript{3} was selected for cotton blended polyester material. Similarly for polyamide and viscose, D\textsubscript{2}C\textsubscript{2} and D\textsubscript{1}C\textsubscript{1} were selected.
In order to increase the wash durability of drug treated textile materials the selected drug-carrier combinations (D$_2$C$_1$, D$_2$C$_3$, D$_2$C$_2$ and D$_1$C$_1$) were encapsulated in a polymer using microencapsulation method. The textile materials treated with the selected drug-carrier combinations showed good resistance to wash fastness till 15 washes. When compared to Reactive dye method the reduction percentage of test bacteria were more in 10$^{th}$ wash for microencapsulated drug treated textile materials.

*Physical, chemical and biological characteristics of biomaterials*

The physical parameters of the reactive drug and encapsulated drug treated fabrics showed significant changes in the tensile strength, fabric weight (GSM) and air-permeability when compared to that of untreated fabrics. The topographical study revealed that the drug-carrier coated biomaterials (silicone and cotton) were smoother than the uncoated materials. The smoothness in the material surface was proposed to be due to the addition of lipid-based carriers. FTIR analysis showed that the significant functional groups of the material, drugs and carriers were not changed in the *drug-carrier coated* materials (silicone and cotton). Functional groups and its peak assignments of each uncoated material (silicone, cotton), drugs (ofloxacin, ornidazole) and carriers (beta-cyclodextrin, DL-lactic acid) were observed. Similar functional groups in the *drug-carrier coated* material proved that the chemical compounds were unchanged. Biocompatibility of the implantable materials and non-implantable materials were tested using HET-CAM method. No signs of visible tissue reactions or degenerative cells were observed on CAM and the surrounding blood vessels.