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Synthesis and characterization of polyether urethane coatings for preventing implant infection

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The common complication associated with implants is microbial infection due to biofilm formation. Among bacterial infections *Staphlyococcus aureus* remains a major challenge. This threat posed by implant associated infection affects a large percentage of population. One of the strategies to combat this risk is to coat the implant surface with polymers loaded with antibiotics. The antibiotics release at implantation site will prevent microbial infection. We have synthesized polyether urethane membrane using biocompatible isocyanate. Synthesis of the polyether urethane membrane was confirmed through Fourier transform infrared spectroscopy analysis. The membranes were further characterized by X-ray diffraction, swelling study (in water, simulated body fluid, tetrahydrofuran), drug release study, and antibiotic assay. Information of swelling study is used for drug loading and explanation of drug release from the membranes. We have used antibiotics for drug release study as they find application to combat infections. From the results, it was observed that antibiotic-loaded implant coatings may find application for preventing implant infection.

Keywords: swelling property; drug release; antibiotic assay; biofilm

1. Introduction

The major complication associated with implants is microbial infection due to biofilm formation. Biofilms are remarkably resistant to both the immune response and systemic antibiotic therapies, and thus their development is the primary cause of implant-associated infection.[1] Once formed, a biofilm is extremely difficult to eradicate, even with vigorous antibiotics treatment.[2] Unfortunately, the lack of a suitable treatment often leaves extraction of the contaminated device as the only viable option for eliminating the biofilm.[1] Therefore, inhibiting biofilm formation is the most crucial step in preventing implant-associated infection. Among different infection-causing bacteria, *Staphylococcus epidermidis* and *Staphylococcus aureus* (gram negative bacteria) are the most difficult ones to handle and are responsible for most of the implant-related infection.[3–5] Development of appropriate biodegradable polymer coating for medical devices to entrap antibiotics, has gained importance in recent research.[6] Antibiotic would release from the coating in the implantation site and this released antibiotic will prevent the bacterial infection. Among different polymers used as implant coating,

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polyurethane elastomers are among the high-performing medical-grade polymers.[7] Chemical, mechanical, and biological properties of polyurethane make them suitable to be used in a diverse range of implantable medical devices. This owes to their unique combination of toughness, durability, flexibility, hydrophobic–hydrophilic balance, biocompatibility, and biostability.[7] Many research activities have been undertaken to resist bacterial adhesion on implant surface, where antibiotic loaded polymer coatings have shown significant preventive strategy by arresting the biofilm formation.[8] Therefore, polymers can be used for coating implants and other medical devices for preventing microbial infection.

We have synthesized a polyurethane coat by using biocompatible isocyanate (PUBI). *In vitro* drug release study was carried out in simulated body fluid (SBF) and water. Antibiotic was loaded in the synthesized polyurethane. Antibiotic-loaded polyurethane membranes were assessed for their capability of preventing microbial growth through antibiotic assay.

2. Experimental

2.1. Materials

Tetrahydrofuran (THF) and Polyethylene glycol (PEG) 400, Merck India Ltd. Hexamethylene diisocyanate (HDI), Sigma–Aldrich. Nutrient broth, Hi media. Bacterial culture (*S. aureus*). Streptomycin, Abbott Healthcare Pvt Ltd. Rifiampicin, Central Drug House, India.

2.2. Method

2.2.1. Synthesis of polyether urethane

The polyurethane membrane was synthesized by reacting PEG with HDI in THF medium at 80 °C for 1 h with controlled and continuous stirring. The reaction scheme is shown in Figure 1. Film was cast by pouring the solution in Petri plates. The membrane was then cured for 5 h at 80 °C in oven and after that swelled in water and THF. Finally, the membranes were oven-dried at 65 °C. Polyurethane membrane samples were washed with water to remove water soluble impurities, if any.

2.2.2. Preparation of SBF

SBF solution has ionic composition and pH almost equal to that of human blood plasma. It does not contain any organic constituents and proteins. There are a large number of existing formulations for SBF. We have used the composition of Hanks' balanced salt solution.[9]

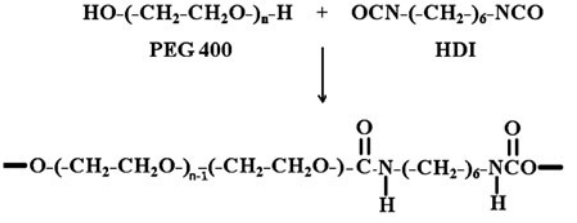

Figure 1. PUBI synthesis scheme.

2.2.3. Bacteria culture media preparation

For culturing bacteria, the media was prepared by dissolving 3.75 g nutrient broth and 5 g agar in 250 ml distilled water. The media was sterilized by autoclaving (15 lb/in2, 121 °C for 15 min).

2.3. Characterization

2.3.1. Swelling analysis

Dried and pre-weighed pieces of polyether membranes were immersed in THF, SBF, and water. The membranes were taken out of solution, gently wiped, and weighed from time to time. The total duration of swelling analysis was six h. The degree of swelling was calculated as follows:

$$\text{Degree of swelling} = \frac{(W_F - W_I)}{W_I} \times 100 \quad (1)$$

where W_F and W_I are the final wet and initial dry weights of the PUBI membranes, respectively.

2.3.2. FTIR analysis

FTIR analysis of PUBI membranes was performed by using Schimadzu FTIR instrument in the frequency range of 3000–500 cm^{-1}. Before FTIR study, the PUBI membrane was prepared by cleaning with acetone to remove any impurity.

2.3.3. Degradation study

Degradation study of the membrane was carried out for 10 days in 20 ml of SBF. Dried and pre-weighed pieces of polyether membranes were immersed in SBF and then incubated at 37 °C. At different time intervals, the samples were taken out. The samples were then wiped, swelled in water, and oven-dried at 65 °C. After drying, the weight of the sample was noted. The weight loss was calculated as follows:

$$\text{Percentage weight loss} = \frac{(W_D - W_I)}{W_I} \times 100 \quad (2)$$

where W_D and W_I are the final and initial dry weights of the PUBI membranes, respectively.

2.3.4. Drug loading

One of the best approaches to improve the efficiency of conventional antibiotics is to release them at the site of implantation from a surface coating. Antibiotics used for studying drug release are streptomycin and rifampicin. Biocompatible polymer coatings (e.g. polyurethane, silicone rubber, polyhydroxyalkanoates, etc.) that actively release antibiotics represent the first class of local antibiotic delivery strategies.[4] The membrane samples were dipped in antibiotic solution to facilitate loading of the drug onto the membrane. Water was used as a solvent for all the loaded antibiotics. Concentration

of antibiotics solution used for loading was 1.2 and 200 mg/ml for rifampicin and streptomycin, respectively.

2.3.5. Drug release study

Drug release study was conducted in both SBF and water medium. At regular intervals, the medium was assayed for drug release using UV–vis spectrophotometer. The amount of drug released was determined spectrophotometrically at 257 nm for rifampicin and 195 nm for streptomycin, with replacement of fresh solvent. The total duration of drug release study was 10 days. The concentration of drug released was determined from the standard plot of absorbance vs. concentration for the respective antibiotics.

2.3.6. X-ray diffraction

X-ray diffraction (XRD) pattern was obtained for PUBI membrane using Rigaku mini-flex X-ray diffractometer, Ultima III with a Cu radiation. The measurements were performed in the 2θ range of 5–60°.

2.3.7. Antibiotic assay

The culture media was autoclaved and poured in Petri dish, and was allowed to solidify. *S. aureus* culture was spread on the media. Membranes loaded with antibiotics were placed in the Petri dish. The antibiotic activity of rifampicin and streptomycin was determined by measuring the diameter of zone of inhibition. The Petri dishes were kept in incubator overnight at 37 °C. The zone of inhibition was recorded for one week. Ten µl of SBF was added on the PUBI membranes in one-day interval to facilitate the diffusion of antibiotic in the surrounding medium.

3. Results and discussion

3.1. Swelling analysis of PUBI membrane

Results of swelling analysis of PUBI membrane are shown in Figure 2. Swelling of polyurethane membrane is higher in SBF than in both water and THF. This may be because SBF has a slight alkaline pH. This slightly alkaline pH of SBF might have triggered degradation resulting in disruption of polymer chains leading to an increase in solubility of polymers.[6] Overall swelling of membrane is (30–32%), (36–37%), and (32–34%) in THF, SBF, and water, respectively.

3.2. FTIR analysis

The synthesis of the PUBI was confirmed by FTIR analysis (Figure 3). There is a strong peak at 1714 cm^{-1} due to the carbonyl group of the urethane bond. Another peak observed at 1642 cm^{-1} is due to a hydrogen-bonded carbonyl group of polyurethane. There is a characteristic peak at 1110 cm^{-1} which confirms the C–O–C aliphatic ether linkage. Antisymmetric, symmetric stretching of C–O provides peaks at 1247 and 1125 cm^{-1} in the FTIR curve. For aliphatic C–H, the stretching band appeared around 2850 cm^{-1}.

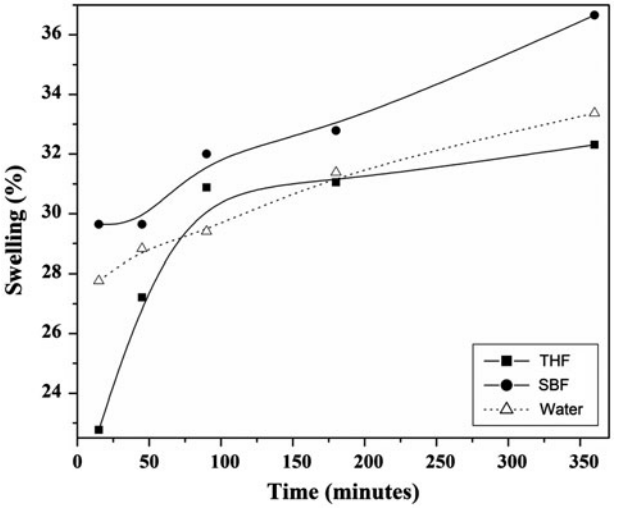

Figure 2. Swelling of PUBI in different medium.

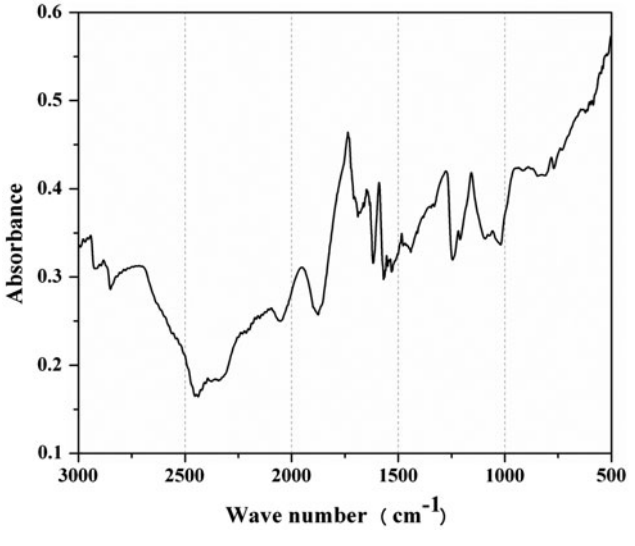

Figure 3. FTIR spectrum of PUBI.

3.3. *Degradation study*

Degradation of PUBI membrane was performed in SBF at 37 °C. The overall degradation of the membrane is 13.48% for a period of 10 days (Figure 4). Since degradation was carried out in SBF, only hydrolytic degradation is responsible for the weight loss of the membrane. The slight alkaline pH of SBF has facilitated the hydrolytic degradation.

3.4. In vitro *drug release analysis*

Figure 5 shows the release of rifampicin and streptomycin over a period of 10 days. It is observed that the release of the drug is more in water than in SBF for both the drugs.

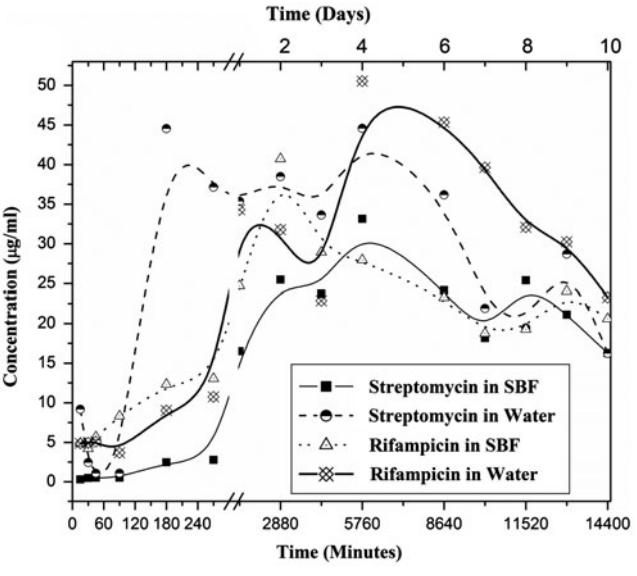

Figure 4. Degradation study of PUBI.

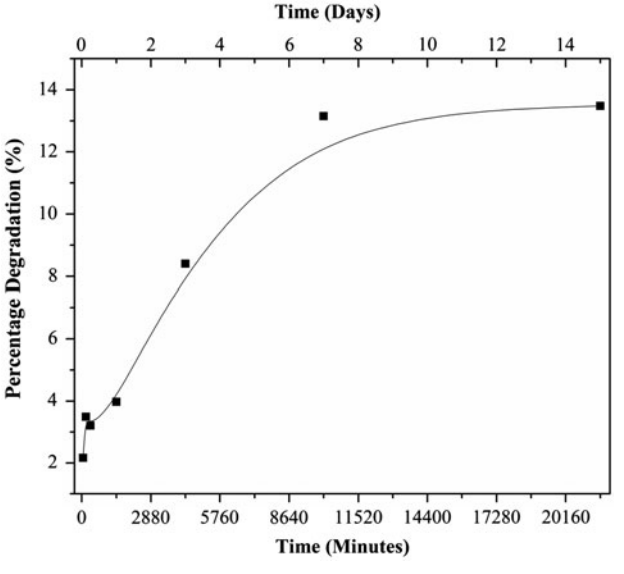

Figure 5. Release of antibiotics from PUBI.

This may be due to the difference in the ionic nature of the drug release medium. SBF is ionic in nature and ions may hinder the release of drug from the membrane, whereas drug diffuses out easily when the medium is water. Again, it is observed that the drug release rate decreased after the rate of degradation became constant (as per Figure 4). So, degradation may have facilitated drug release. However, antibiotic concentration in the release medium is more than its minimum effective concentration (MIC) (MIC for rifampicin and streptomycin are 1 and 16 µg/ml, respectively), so they will be effective in the inhibition of bacterial infection.[10,11]

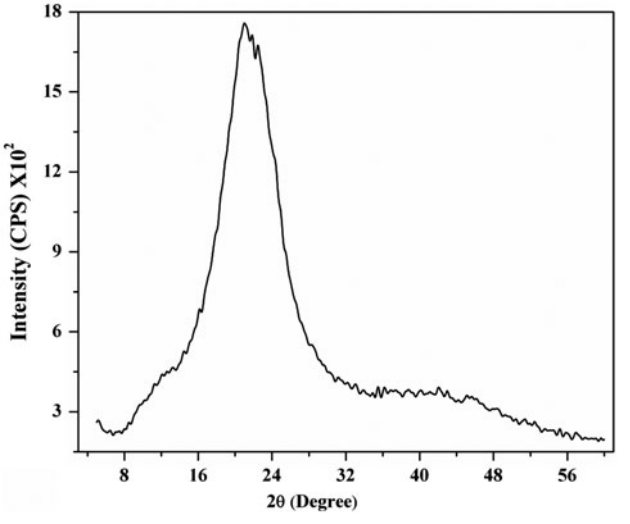

Figure 6. XRD pattern of PUBI.

3.5. XRD analysis

XRD pattern is shown in Figure 6. From the pattern, it appears that the PUBI membrane is amorphous in nature. During curing, cross-linking of the molecules is rapid and in a disordered fashion which facilitates the formation of amorphous region in the membrane.

3.6. Antibiotic assay

Antibiotic assay was carried out using rifampicin and streptomycin. Higher zone of inhibition was observed for rifampicin than for streptomycin as shown in Figure 7. Hence, rifampicin activity against the bacterial growth is better than streptomycin when

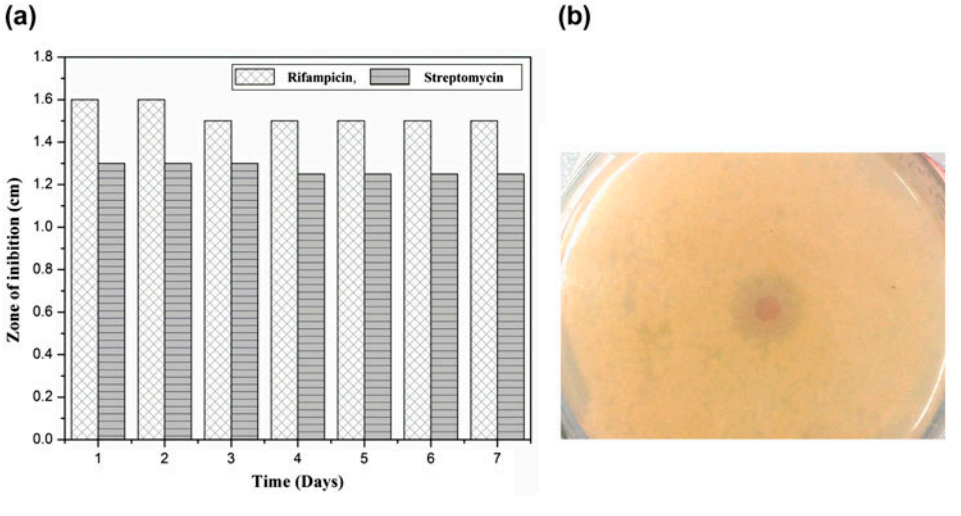

Figure 7. (a) Zone of inhibition of rifampicin and streptomycin. (b) Pictorial diagram of antibiotic assay (rifampicin).

loaded in the PUBI matrix. Zone of inhibition was maintained for one week's time with a small decrease in the diameter of inhibition after two days, for both the antibiotics. As SBF was added to PUBI, it helped in proper diffusion of antibiotics in the surrounding medium. Due to this, zone of inhibition has been maintained for one week's time.

4. Conclusion

In summary, HDI-based polyether urethane was successfully synthesized. FTIR study confirms the synthesis of polyurethane bond. Swelling study reveals that this HDI-based polyurethane has similar swelling in all the three mediums, namely SBF, THF, and water. Both the drug release study and antibiotic assay were carried out for these PUBI membranes. From the results, it was observed that the loaded antibiotics had prevented the bacterial growth. So, these membranes can be used as a matrix for antibiotic loading to prevent bacterial infection. These membranes loaded with antibiotics should be subjected to antibiotic assay for a longer duration before going for animal trial. Degradation study reveals that the PUBI membranes were degraded in SBF. Hence, it can be concluded that the polyurethane membrane has the potential to be used as an implant-coating material to prevent biofilm formation, and due to biodegradable nature it will be excreted from the body slowly with time.

Acknowledgments

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