Biocompatibilities of Some Synthetic Polymers in Films

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ABSTRACT

We chose two synthetic polymers (poly(vinylidene fluoride-*co*-hexafluoropropylene) and Nafion-117®) and studied their biocompatibility to several bacteria that are most notorious for opportunistic and iatrogenic infections.

Keywords: fluorinated polymers, biocompatibility, adhesion, surface characteristics, chemical composition

1 INTRODUCTION

Along with a rapid progress in medicine, the field of organ transplantation has been dramatically expanded. However, due to the shortage of supplies from organ donation, artificial organs and prosthetic implants have gained great attention from both academia and biomedical industry. For artificial organs and prosthetic implants, synthetic and natural polymers have been used over the last thirty years. One of the most serious clinical complications associated with the use of these biomaterials is bacterial infection [1-3]. Since biomaterial-related infections prove extremely resistant to antimicrobial therapy, the resolution in most cases would require the removal of the implanted devices. The common feature of these infections involves the adhesion of bacteria to the biomaterial surface followed by colonization and biofilm formation. Therefore, the early stage of bacterial adhesion to the biomaterial surface is the critical event in the pathogenesis of foreign body infection. *Pseudomonas aeruginosa* (*P. aeruginosa*) is frequently isolated from infections associated with artificial hip prostheses, central venous catheters, urinary catheters, and extended-wear contact lenses. *Staphylococcus epidermidis* (*S. epidermidis*) and *Staphylococcus aureus* (*S. aureus*) are other organisms that commonly cause infections associated with prosthetic joints, central venous catheters, intrauterine devices, and prosthetic heart valves. *Enterococcus faecalis* (*E. faecalis*) can cause diseases of the urinary tract and subcute bacterial endocarditis and *Escherichia coli* (*E. coli*) has also been identified as a significant cause of urinary catheter infections. Cell adhesion to other cells and to various substrates, such as the extracellular matrix or other artificial supports, is a critical process in the formation of tissues, cell differentiation, and morphogenesis [4,5]. Cell adhesion to foreign surface is also important in the biocompatibility of implants and in the growth of anchorage-dependent cells in culture. The ability to control cell-surface interactions is of paramount importance in controlling host-biomaterial interactions, in predicting cell behavior in cell engineering, in understanding tissue development, as well as in realizing the potential to tissue engineer solid organs. In order to identify physicochemical factors of the polymeric materials engaged in early stage of bacterial adhesion, we investigated adhesion of *P. aeruginosa*, *S. epidermidis*, *S. aureus*, *E. faecalis*, and *E. coli* to films of poly(vinylidene fluoride-*co*-hexafluoropropylene) (PVFHFP), and Nafion-117® ionomer that reveal different chemical and physical characteristics.

2 EXPERIMENTAL

PVFHFP (average-weight molecular weight 400,000 and polydispersity index 3.08) was purchased from Aldrich Chemical Company, which is a semicrystalline polymer that reveals a melting temperature T_m of 143 °C. Nafion-117® in films with 178 μm thickness was also obtained from Aldrich, which is a hygroscopic polymer bearing sulfonic acid groups as an ionomeric component. PVFHFP were molded at 150 °C under nitrogen atmosphere, as films with around 150 μm thickness, using a hot-pressure system. In addition, Nutrient broth (NB) and NB agar plates were purchased from Difco Company. Other chemical compounds, including Tween-20® (polyoxyethylene(20) sorbitan monostearate), sodium hydrogenphosphate and sodium dihydrogenphosphate, were purchased from Aldrich. Phosphate-buffered saline (PBS, pH 7.4) was made from sodium hydrogenphosphate and sodium dihydrogenphosphate. *P. aeruginosa*, *S. epidermidis*, *S. aureus*, *E. faecalis*, and *E. coli* were obtained from the Korean Culture Center of Microorganisms and routinely grown in NB or on nutrient agar plates (NAP). A single colony of each bacterium was inoculated into 5 mL NB and incubated overnight at 37 °C with shaking (250 rpm). The overnight culture was then diluted 100-fold with NB and further incubated at 37 °C with shaking until a mid-logarithmic phase was reached. The bacterial culture was centrifuged and then the supernatant was removed. The bacterial pellet was resuspended in PBS and used for adhesion experiments. For bacterial counting, the bacterial suspension in PBS was serially diluted with PBS, plated on NAP, and incubated overnight at 37 °C. The bacterial suspension was adjusted to $1.20 \times 10^6 - 1.93 \times 10^6$ CFU (colony forming units) per mL.

Film specimens (2 × 2 cm) of each polymer were immersed in 70% ethyl alcohol for 5 min and then dried aseptically in air. Each film specimen was put into 10 mL PBS in a 50 mL cornical tube. One hundred μL of the bacterial suspension was added into the tube and incubated at 37°C for 4 h with gentle shaking (200 rpm). After the incubation, the film specimen was rinsed in PBS several times to remove the non-adherent bacteria. The rinsed film specimen was transferred into a new tube with 5ml PBS containing 0.05 wt% Tween-20 and sonicated for 5 s twice at 37 °C to detach the adherent bacteria from the film surface. The detached bacteria were counted on NAP.

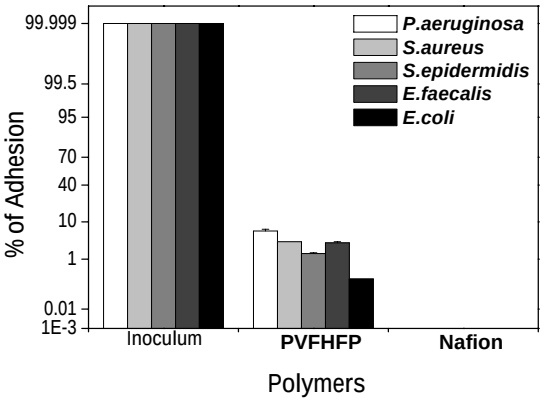

Figure 1. Bacterial adhesion on polymer films

3 RESULTS AND DISCUSSION

For the polymer films, water absorption was examined in water at room temperature and in PBS at 37°C and water wettability also measured. PVFHFP films show no absorption in water and PBS. However, Nafion-117® films exhibit to absorb water very quickly and their water absorptions vary in a range of 7-18 wt%, depending upon the water versus PBS, temperature, and time. These high water absorptions of Nafion-117® film are attributed to the hygroscopic sulfonic acid groups in the polymer. For dried films, water contact angle was determined to be in the decreasing order Nafion-117® film > PVFHFP film. This result indicates that water-wettability is in the increasing order Nafion-117® film < PVFHFP film. The water contact angles of PVFHFP films were found to change very little even after immersed in PBS at 37 °C for 4 h. On contrary, the water contact angle of Nafion-117® film was found to be significantly reduced to 88.0° from 106.0° after immersed in PBS at 37 °C for 4 h. For Nafion-117® films immersed in water at room temperature for 4 h, similar contact angle change was observed (data not shown). The reduced water contact angle of the PBS- or water-treated Nafion-117® film is comparable to that of PVFHFP film. Taking the contact angle changes into account, the water-wettability of Nafion-117® film is increased by the immersion in the PBS solution, becoming comparable to that of PVFHFP film. As described above, Nafion-117® film shows relatively high water absorption capability because of its sulfonic groups. Thus, the reduction of water contact angle observed in the PBS treated Nafion-117® film is attributed to the water absorption of the film during the immersion in the PBS solution. For the polymer films, surface energies were measured before and after immersion in PBS at 37°C for 4 h. The dry Nafion-117® film exhibits the lowest surface energy. Here it is noteworthy that the surface energy of the Nafion-117® film absorbed water is still lower than that of the dry PVFHFP film.

Taking these results into account, the films' biocompatibilities were investigated for several bacteria. The determined results are shown in Figure 1. As can be seen in Figure 1, the Nafion film reveals no adherence in all the five bacteria, while the PVFHFP film exhibits some adherence in all the bacteria. *E. coli* adherence to the film is very poor. The other bacteria reveal some adherence to the PVFHFP film. For these bacterial adhesion results, detailed discussions will be given.

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