Biocompatibilities of Some Synthetic Polymers in Films

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Introduction

Along with a rapid progress in medicine, the field of organ transplantation has been dramatically expanded. Due to the shortage of supplies from organ donation, however, artificial organs and medical implants are receiving high attention. Materials for artificial devices require biocompatibility with the human body. Moreover, it has been well known that the implants are facilely tainted with pathogenic bacteria during surgical processes and that a few bacteria circulate in bloodstream, adhere and start to colonize on the surface of prostheses [1]. Several factors that cause these problems include chemical and physical properties of artificial materials and characteristics of bacteria [2].

In the present study, we chose five different sorts of bacteria, *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Enterococcus faecalis* and *Escherichia coli* that are most notorious for opportunistic and iatrogenic infections, and a human cell, HEp-2 cell which is laryngeal carcinoma, and then investigated their adherence behaviors onto film surfaces of Nafion®, poly(propylene carbonate) (PPC) and poly(vinylidene fluoride-*co*-hexafluoro-propylene) (PVFHFP) that reveal different chemical and physical characteristics.

Experimental

Polymer film preparations. Nafion® and PVFHFP ($\overline{Mw}$ = 400,000) were purchased from Aldrich Chemical Company while PPC of $\overline{Mw}$ = 673,000 was synthesized as described elsewhere [3]. Films of PPC and PVFHFP were prepared by hot-press molding at 110°C and 170°C and Nafion was prepared by it self respectively, under nitrogen atmosphere. Before use for bacterial adhesion, the prepared polymers films were characterized using atomic force microscopy (AFM), water and ethanol swelling analysis, water contact angle analysis, and surface energy analysis.

Bacterial adhesion. *S. aureus, S. epidermidis, P. aeruginosa E. faecalis, and E. coli* were obtained from the KCCM routinely grown in Nutrient broth (NB) or on NB agar plates. NB was inoculated with a single colony of each bacterium on NB agar plate and incubated with shaking at 37°C overnight. The overnight cultured bacterium was then diluted 100-fold and incubated at 37°C with shaking until a mid-logarithmic phase was reached. The bacterial culture was centrifuged, rinsed in phosphate-buffered saline (PBS), and used for adhesion experiments. Serially diluted cultures were also plated for counting on NB plates. Then, each polymer sheet was immersed in PBS and each bacterium of a certain amount was added to the PBS containing the polymer sheet, followed by incubation with shaking at 37°C for 4 h. The polymer film was taken out and rinsed 5 times with PBS, transferred into a tube containing Tween-20 in PBS, followed by sonication. After the film was taken out, the detached bacteria were serially diluted and plated on NB agar plates for counting their population.

HEp-2 cell adhesion. HEp-2 cell line was purchased from KCLB. Cell growth was recorded using an optical microscope (Nikon Coolpix5400) equipped with a digital camera. These three polymer films were cut into squares (1 x 1 cm) and were sterilized by 70% ethyl alcohol for 20 min and then washed in Dulbeccos' PBS solution (pH 7.4) for 2 h. HEp-2 cells were added to the polymer film in the T-25 culture flask; the medium was changed every other day until confluent monolayer was obtained.

Results and discussion

Polymer film properties. Film surface was examined by AFM; the root mean squared roughness was 2-8 nm, depending on the polymer film. Nafion film showed high swelling in the buffer solutions, which results from the ionic characteric due to its sulfonic acid groups. In contrast, both PVFHFP and PPC revealed poor swelling in the buffer solutions.

Bacterial adhesion. The bacterial adhesions were found to be in increasing order Nafion film < PPC film < PVFHFP film. These results indicate that bacteria show a strong tendency to adhere more onto hydrophobic polymer film surface; such tendency is decreased with increasing hydrophilicity and ionic strength on the polymer film surface.

Cell adhesion. HEp-2 cell showed to favorably adhere on the Nafion and PVFHFP films (Figure 1). However, surprisingly the cell did not adhere on the PPC film (Figure 1). The results suggest that HEp-2 cell favorably adheres on the hydrophilic and ionic polymer film surface as well as on the highly hydrophobic polymer surface.

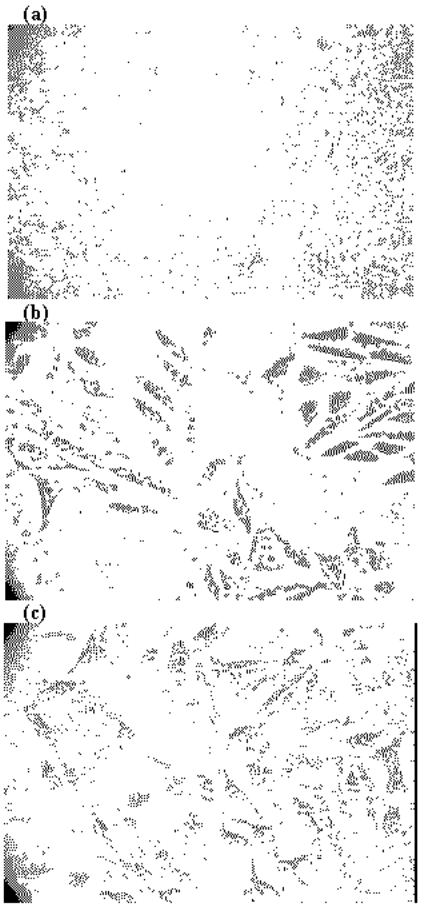

Figure 1. Optical microscopy images of Hep-2 cells on polymer films: (a) PPC; (b) Nafion; (c) PVFHFP.

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