行政院國家科學委員會專題研究計畫成果報告

不同薄膜之表面特性與生物適應性之影響

計畫編號：NSC 87-2314-B-002-075
執行期限：86年8月1日至87年7月31日
主持人：林東燦　　執行機構及單位名稱：台灣大學醫學院檢驗醫學科

ABSTRACT

Monocyte adhesion and subsequent activation are major events that facilitate the foreign-body reaction. These studies evaluate the effect of semicrystalline polyamide (Nylon-66), poly (ethylene-co-vinyl alcohol) (EVAL), and poly (vinylidene fluoride) (PVDF) with nonporous and porous morphologies on the ability of monocyte adhesion and activation to produce variable levels of IL-1β, IL-6, and TNF-α. Results indicated IL-1β was produced in the greatest quantity by these polymers. In addition, monocyte adhesion and activation on a material may alter to a great extent dependent on the surface morphology and wettability. As the membrane wettability increases, the ability of the membrane to adhere monocytes increases but to stimulate monocyte production of cytokines decreases. Similarly, these membranes when prepared with porous surfaces can enhance monocyte adhesion and suppress monocyte activity. Therefore, the nonporous PVDF membrane is the least biocompatible in this work. In contrast, the hydrophilic membrane Nylon-66 with porous surface is the least stimulating of monocyte cytokine production when compared to all of the other membranes evaluated with nonporous or porous surface. These studies provide important insight into conditions that modulate monocyte activity in response to the substratum morphology and wettability.

Keywords: surface morphology, surface wettability, monocyte, adhesion, activation.

INTRODUCTION

The recent progress in biomaterials has raised the monocyte is the major cell type found on the surface of implanted biomedical polymers.[1] Cytokines, such as IL-1β, IL-6 and TNF-α, released by activated monocytes can regulate fibroblasts growth and induce other cells, such as T lymphocytes to proliferate, synthesize proteins and secrete factors further activating macrophages to result in the so-called whole body inflammatory response.[2] Since cytokines contributes to the inflammatory response and may augment an inflammatory response, monocytes on the surface of biomaterials play an important role in the foreign body reaction. Considering these reasons, it was of interest to evaluate the production of cytokines by monocytes in contact with biomaterials. In this work, the combined effect of polymer substratum morphology and wettability on the monocyte (macrophage) adhesion and activation was examined. Monocytes were cultured on three crystalline polymer membranes with nonporous and porous surfaces. Monocyte adhesion was studied by using MTT assay to evaluate cell viability. Monocyte activation was expressed as IL-1β, IL-6 and TNF-α secreted by per MTT conversion.

MATERIALS AND METHODS

Membrane preparation and characterization

The membrane materials used in this study were poly(ethylene-co-vinyl alcohol) (EVAL, E105A containing ca. 56 mole % vinyl alcohol, Kuraray, Japan), polyamide (Nylon-66, Zytel 101 DuPont) and

poly(vinylidene fluoride) (PVDF, Kynar 740, Elf Ato Chem). An appropriate amount of polymer was dissolved in solvent to form a 25 wt.% of polymer solution. The solvent for EVAL, Nylon-66 and PVDF was DMSO, formic acid and DMF, respectively. Nonporous membranes were prepared by evaporating solvent of the casting solution in a vacuum oven at 60°C for 2 days. Porous membranes were prepared by immersing the casting solution into 1-octanol precipitation bath for 2 days. After the evaporation and precipitation were completed, the membranes were soaked in ethanol to remove 1-octanol and residual DMF and then kept in a water bath at 25°C.

The morphology of the membrane was examined using a scanning electron microscope (SEM). The freeze-dried samples were sputtered with gold and palladium in a vacuum and using a Hitachi S-800 microscope at 20 kV. Air-water contact angles were measured at 25°C using a reverse air-bubble apparatus (CA-D, Kyowa Scientific Co.). Six measurements were made for membranes with the nonporous morphology.

Monocyte culture

Human peripheral blood mononuclear cells were isolated by Percoll density gradient sedimentation procedure.[3] The monocyte purity was > 85% as determined by trypan blue dye exclusion. Circular samples (1.5 cm in diameter) were cut from the prepared membranes, rinsed extensively with distilled water and sterilized under ultraviolet light overnight. Subsequently, disks of each test membranes were placed in the bottom of each well of a 24-well tissue culture plate (Corning, New York, USA). Then RPMI-1460 medium with freshly isolated monocytes at a concentration of 1×10^5 cells/well were added to the culture wells. In addition, lipopolysaccharide (LPS, 5 μg/mL) was added to mimic the foreign-body reaction seen at implanted biomaterial surfaces in vivo. Cell culture was maintained in a humidified atmosphere with 5% CO_2 at 37°C.

MTT assay

A colorimetric assay using the MTT assay is being increasingly used to measure viable cell number after release of cytoplasmic contents into the medium from artificially lysed cells.[4] MTT (0.35ml) was added to each well and plats were incubated for 5 h at 37°C. After incubation, the medium was aspirated and the formazan reaction products were dissolved in 0.4 ml of 10% sodium dodecyl sulphate (SDS, Sigma) in PBS and the plates were then shaken for 15 h. The optical density of the formazan solution was read on an ELISA plate reader (ELx 800, BIO-TEK,) at 570 nm. Viable cell number determined by the MTT assay was performed at 24 h after plating. All experiments were repeated four times.

Cytokine assay

After 24 h incubation, supernatant was harvested and cytokine (IL-1β, IL-6 and TNF-α) concentration released into the medium was measured by ELISA procedure following the manufacturer's directions (Endogen, Inc. Boston, MA). All experiments were repeated four times.

RESULTS

Membrane morphology

Macroscopically, nonporous membranes were transparent and porous membranes appeared opaque. The microscopic analysis of membrane surface morphologies was evaluated by using SEM. Nonporous membranes prepared by solvent evaporation had similar morphological appearance of surface, with a dense and smooth structure. On the other hand, it is interesting that even though these polymers have rather different chemical properties, they all form membranes with particulate morphology as they are precipitated from 1-octanol. The diameters of particles were estimated approximate 0.3μm, 4μm and 1μm for membranes EVAL, Nylon-66 and PVDF, respectively.

Contact angle analysis

Air-water contact angles were 22.1±2.4°, 56.1±1.8,° and 132.2±2.4° for Nylon-66, EVAL and PVDF, respectively. This shows that Nylon-66 and PVDF was relatively the most hydrophilic and the most hydrophobic membrane.

Monocyte adhesion

Figure 1 shows the formazan accumulation for the membranes and control at 24 h after plating. Data in Figure 1 indicate the correlation of membrane water contact angle on monocyte adhesion. As the membrane water contact angle decreases, the ability of the membranes to adhere monocytes increases regardless of the nonporous or porous surfaces. For nonporous membranes, PVDF inhibited monocyte adhesion compared to controls and EVAL indicated a slight higher formazan absorbance than controls. Only Nylon-66 had the significant difference of accumulation of formazan compared to controls ($p < 0.05$). In contrast to nonporous membranes, three porous membrane had higher accumulation of formazan and significant differences compared to controls ($p < 0.05$). This indicates that a membrane with porous morphology was favorable for the attachment of monocytes regardless of membrane water contact angle.

Cytokine production

Table 1 presents the amounts of IL-1β, IL-6 and TNF-α detected in the monocyte derived culture supernatants after 24-h incubation. When the data are evaluated based on cytokine dependence, the produced cytokines in quantities following the order: IL-1β > IL-6 > TNF-α. In addition, studies have shown that polymer surface hydrophilicity and morphology may alter the ability of monocytes to adhere but it may not influence the release of cytokines from monocytes. The level of cytokine production in the monocyte culture supernatants for various membranes with porous or nonporous morphologies was similar except porous EVAL and Nylon-66 membranes show significant inhibitory effect on TNF-α release compared to controls ($p < 0.05$).

Evaluation was taken one step further based on the influence membrane surface properties on the monocyte activity per monocyte adhering to the membranes. Monocyte activity was obtained by evaluating the production of cytokines divided by MTT conversion, i.e., the cytokine release per monocyte. This more comprehensive view of the effect of membrane surface property in monocyte activity is presented in Figure 2. It clearly shows that porous membranes had lower monocyte activity and significant differences compared to controls ($p < 0.05$) regardless of the wettability of polymer. Furthermore, the monocyte activity in vitro monocyte culture was PVDF > EVAL > Nylon-66 regardless of the nonporous or porous surfaces. Based on our water contact angle results, monocyte activity may be in a direct relationship with surface wettability. This suggests that as the membrane water contact angle increases, the ability of the membrane to stimulate monocyte production of cytokines increases. Therefore, the nonporous PVDF membrane is the least biocompatible in this work. In contrast, the hydrophilic membrane Nylon-66 with porous surface is the least stimulating of monocyte cytokine production when compared to all of the other membranes evaluated with nonporous or porous surface.

DISCUSSION

It is obvious from this study that different membrane surfaces are important in modulating monocyte adhesion and activation. When the nonporous PVDF membrane was subjected to monocytes in vitro for 24 h, it showed the greatest inhibition of monocyte adhesion compared to other membranes. However, it showed the greatest cytokine production per activated monocyte. This strongly suggests that the more reactive surface would activate cells to elicit a greater foreign body reaction, but it is

not certain to attach more monocytes. Therefore, nonporous membranes attract fewer adherent monocytes than porous surfaces, whereas every monocyte on nonporous membranes releases higher amounts of cytokine than on porous membranes. Based on this result, interaction of monocytes with the membrane surface consists of two distinct successive stages. The first stage is monocyte adhesion, where some interactions are between monocytes and the membrane surface. Surfaces eliminating or minimizing this interaction between monocytes and membranes would be expected to inhibit monocyte adhesion. Hydrophobic PVDF is a candidate for this type of material. The stage of monocyte adhesion is followed by the second stage: monocyte activation. In this activation stage, reorganization of intracellular cytoskektal components (microfilaments and microtubules) takes place due to changes in the energy metabolism of monocytes. Thus, monocytes undergo shape change and release of cytokines, which helps provide the impetus to inflammation. Even though a limited number of monocytes are adhered, every monocyte may secrete a great amount of cytokines because every monocyte is highly activated.

REFERENCES

1. B. Ranter, A. Johnston, and T. Lenk, "Biomaterial surfaces," *J. Biomed. Mater. Res.*, **21**, 59-87, (1987).
2. M.A. Cardona, R.L. Simmons, and S.S. Kaplan, "TNF and IL-1 generation by human monocytes in response to biomaterials, J. Biomed. Mater. Res., 26, 851-859 (1992).
3. H. Pertoft, A. Johnsson, B. Warmegaro, and R. Seljeld, "Separation of human monocytes on density gradients of percoll", *J. Immunol. Meth.*, **33,** 221-229 (1980).
4. T. Mosmann, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, J Immunol Methods, 65 (1983) 55-63.

Table 1: Cytokine production on various membranes (n=4).

Sample	IL-1β		IL-6		TNF-α	
	Nonporous	porous	Nonporous	porous	nonporous	porous
PVDF	989±45*	966±50	704±75	696±11	260±65	220±85
EVAL	885±36	812±51	744±40	662±13	173±23	71±17*
Nylon-66	862±37	654±26	685±35	623±32	150±20	23.5±18*
Control	861±68		753±75		209±56	

Figure 1: MTT conversion for various membranes (n=4).

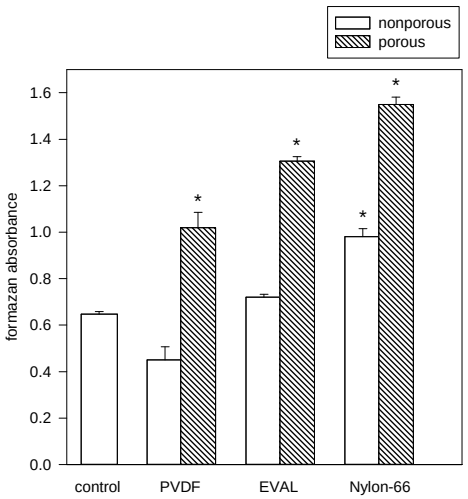

Figure 2:Monocyte activity on various membranes (n=4).