Grafting PS with Ammonia and Polylsine by Plasma and the Interaction with Different Cells

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Abstract In tissue engineering materials area the treatment of materials surface in important for the cells to adhere. And the plasma is used generally to introduce functional groups onto the surface, but seldom research on grafting amino acid by plasma has been carried out. In this research the cell culture plate(polystyrene) was induced ammonia and polylsine by low temperature plasma. And the treated plate showed good property for the cells to adhere and no toxicity to cells compared with untreated and commercial plates. So grafting ammonia and polylsine onto surface of cell culture plate by plasma is a simple, economical and efficient method to advance the nature of cell culture plate.

Keywords: plasma; polylsine; biocompatibility

Introduction

Tissue engineering materials are paid more attentions to along with the development of cell culture technology and imitation artificial tissue and organ of the tissue engineering. Researching the materials that have good tissue compatibility is the basement [1 ~ 3]. The interaction between cells and materials is the key problem beacuse the adhering of cells to materials is the premise of proliferation, differentiation and proliferation. In fact the interation between cells and materials is the receptors of cells recognizing the groups of material surface and cell adhesion and growth on a surface is influenced by the local physical and chemical environment of the matrix. Up to now the materials used are biologically inert and lack of groups to conjunct specially with cells receptors so the materials can not provide perfect matrix for cells to adhere to. [4 ~ 8]. An effective method to solve this problem is treating the surface of the materials to generate functional groups such as galactose without changing of the nature and mechanical strength of the materials[9 ~ 12].

Here we report a simple method to treat the cell culture plate with plasma gun. Compared with untreated control the treated cell culture plate show good histocompatibility and provide better matrix for cells to attach.

1. Materials and methods

1.1. Materials
Polystyrene plates were proved by Long-Chuan biotech. And the control cell culture plates was purchased from Corning Incorporated, RPMI-1640 was purchased from Gibco (Gaithersburg, MD) 3-(4,5)-dimethylthiahiazoro (-z-y1)-2,5-diphenytrazolumromide (MTT) was purchased from Sigma. trypsin was purchased from Amresco. Pregnant mouse was purchased from Center for New Drugs Evaluation of Shandong University (Shandong, China). All other chemicals were of analytical grade.
1.2. Methods

1.2.1. Treating of the cell culture plate

The cell culture plates were treated in vacuum degree -0.1Pa environment with atomized ammonia and polylysine for 10 seconds. Then the plates were sterilized by gamma radiation.

1.2.2. Velocity of cells sticking to the wall

Fetal mice of 15 days were obtained by an open surgery on the pregnant mouse under ether anesthesia. And the skeletal muscle, brain and intestinal of fetal mice were crushed in cutting technique and trypsinization. The single cells were washed with PBS three times. Then the same quantity of cells were inoculated into treated, untreated and control cell culture plates separately then cultured in 37℃ and 5% CO₂ and the morphological and adhesive changes and the amount of the cells were observed under an inverted microscope after 3 hours. 48 hours latter the cells were washed three times gently with PBS and 200 μL 0.01% neutral red per well was added to stain the cells in 37℃ for 1 hour. After washed three times with PBS gently, 100μL destaining solution per well was added and absorption value (A540) was tested. The data was analysis with SPSS13.0.

1.2.3. Efficiency of cells sticking to the wall

The MA104 cells were trypsinized into single cells then 1500 cells (200μL) per well were inoculated into treated and untreated cell culture plates separately and cultured in 37℃ and 5% CO₂. The cells had adhered were counted and data was washed three times gently with PBS and 200 μL 0.01% neutral red per well was added to stain the cells in 37℃ for 1 hour. After washed three times with PBS gently, 100μL destaining solution per well was added and absorption value (A540) was tested. The data was analysis with SPSS13.0.

2. Result

2.1. Velocity of cells sticking to the wall

There are more cells in treated cell culture plate has adhered to the wells and show better growth state than those in untreated and controls in 3 hours (Table1). After 48 hours the value of A540 of treated group is 1.597±0.16 which is much higher than those of untreated and control groups(Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>treated</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>intestinal</td>
<td>56.71±18.53</td>
<td>161.37±60.99*#</td>
<td>152.00±28.68*</td>
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<tr>
<td>Skeletal muscle</td>
<td>37.50±22.93</td>
<td>138.17±31.76*</td>
<td>166.00±41.08*</td>
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<tr>
<td>brain</td>
<td>8.67±1.21</td>
<td>41.17±10.94*#</td>
<td>15.83±7.25*</td>
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*P<0.01 VS untreated group  
#P<0.05 VS control group  
@P<0.01 VS control group

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>treated</th>
<th>control</th>
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</thead>
<tbody>
<tr>
<td>0.452±0.12</td>
<td>1.597±0.16*@</td>
<td>1.388±0.13*</td>
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</table>

*P<0.01 VS untreated group  
@P<0.01 VS control group

2.2. Efficiency of cells sticking to the wall

The amount of adhered cell of treated group is 208.17±10.76 which is much higher than those of untreated and control groups and the difference has statistical significance(Table 3). The mean value of A540 of treated group
is 0.539±0.17 and 0.721±0.22 after 15 and 48 hours separately those are also has statistical significance compared with untreated and control groups (Table 4).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>The amount of cells adhered after 8h (X±SD, n=10)</th>
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<tbody>
<tr>
<td></td>
<td>Untreated</td>
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<tr>
<td></td>
<td>50.17±15.82</td>
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*P<0.01 VS untreated group  
@P<0.01 VS control group

<table>
<thead>
<tr>
<th>Table 4</th>
<th>The absorption value of A540 (X±SD, n=10)</th>
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<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td></td>
<td>15h 48h</td>
</tr>
<tr>
<td>intestinal</td>
<td>0.539 0.721</td>
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<tr>
<td></td>
<td>±0.17 ±0.22</td>
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<tr>
<td>Skeletal muscle</td>
<td>0.652 0.815</td>
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<td>±0.18 ±0.26</td>
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<tr>
<td>brain</td>
<td>0.175 0.128</td>
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<tr>
<td></td>
<td>±0.15 ±0.14</td>
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</tbody>
</table>

*P<0.01 VS untreated group  
@P<0.01 VS control group

3. Discussion

Low temperature plasma is composed of positive and negative charged particle and neutral particle. It can excite, dissociate and ionize the molecular on the surface of treated materials followed by breaking or and forming chemical bonds. It can erose surface and form crosslinking with introducing some functional groups such as -NH2. By the extra functional groups the surface would be modified[13] and different functional groups proved different properties[14~17]. This technology of surface modification has been used in biology area since 1980s and most attentions were payed to artificial implanting material. the plasma-treated segmented-polyurethane (SPU) surface was changed in wettability which improved the bovine aortic endothelial cells (BAEC) proliferation and adhesion it suggest that the plasma treatment would be useful for developing a small-calibre hybrid vascular graft[18]. plasma-treated polyethylene terephthalate (PET) samples showed an increase in cell growth with incubation time and the presence of well-spread and flattened cells in cytocompatibility tests. So it can be said plasma treatments can improve PET biocompatibility. More importance is the plasma-treated PET has no toxic effect on human umbilical vein endothelial cells (HUVEC)[19].

In this research we treated the cell culture plate with plasma to graft ammonia and polylysine onto the surface and estimated its biocompatibility. In the experiment of culturing primary cell the treated cell culture plate show better biocompatibility and the cells adhered much earlier than the untreated group in 3 hours. There were more cells adhered in treated group in three different cells which show that the plate treated grafted with ammonia and polylysine is suit for multiple cells. So the modification of cell culture plate surface with low temperature plasma leads to the facilitation of cell adhesion. Compared with the commercial the treated plate also showed property in culturing the cells that difficult to cultivate such as brain. In the followed experiments the date also showed the treated plated has superiority.

In summary, the cell culture plate treated with plasma got a good property for cells adherent control with untreated and commercial control groups.
Reference