2ESB03

Development of cell rolling column modified with betain polymers

Hao CHEN[1], Atsushi MAHARA[1], Carlos AGUDELO[1], Hiromi KITANO[2], Tetsuji YAMAOKA[1]

([1]Department of Biomedical Engineering, National Cerebral and Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita 565-8565 Japan, [2]Department of Applied Chemistry, Graduate School of Science and Engineering, University of Toyama 930-8555 Japan)

[1]Tel: +81-6-6833-5012 ext2637, Fax: +81-6-6835-5476, E-mail: yamtet@ri.ncvc.go.jp

Key: Biomaterials/Cell Rolling/Amphiphilic Polymer

[PREFACE]

Human ES and iPS cell research increased remarkably in recent years since separation of single type cells is a very important issue for these stem cell researches.

An efficient separation system is needed to isolate the specific cell population. The surface marker of the cells has been using for the conventional cell separation. However, there is a problem that in almost cases, the cells have to be labeled with antibody and it would contaminate the cells. In the previous study, our group has focused on the cell rolling and developed the cell separation silicon column and glass column with the antibody immobilized interface through the poly(sulfopropyl betain) brush[1, 2]. However, the polymerization should be performed in the column. In this study, we synthesized an amphiphilic copolymers composed of 2-methacryloyl oxyethyl phosphorylcholine (MPC) as hydrophilic segment and *n*-butyl methacrylate (*n*BMA) as hydrophobic segment (Figure1 (a)) and coated the column surface via hydrophobic interaction. The antibody was immobilized on the surface of micro-chamber that has a vertical crossed flow channel. The cells were injected and settled out, and cell rolling behavior was evaluated.

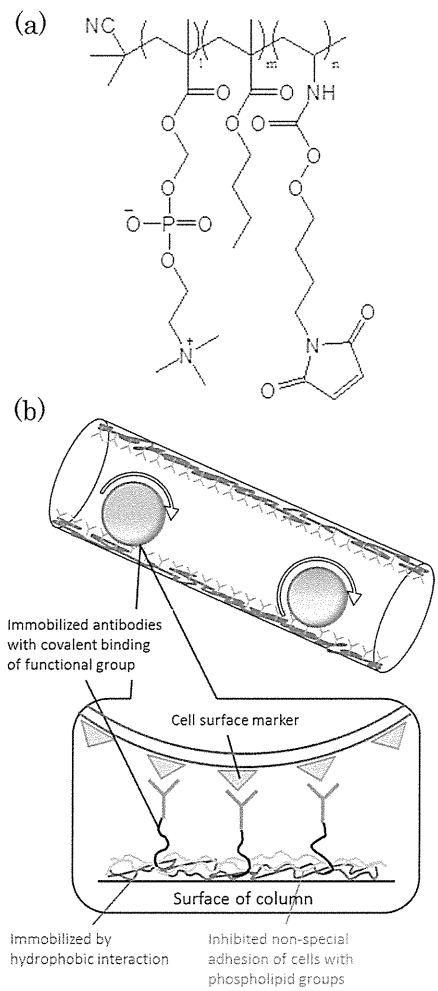

Figure.1 (a) Chemical structure of MPC-*n*BMA-NVA and (b) cell rolling on the polymer coated surface.

[RESEARCH]

The *random*-copolymers were synthesized by free radical polymerization of MPC that has betain group, *n*MBA, and *N*-vinylformamide(*N*VFA) (MPC-*n*BMA-*N*VFA). The poly(*N*VFA) segment was changed to primary amino

group by hydrolysis with hydrochloric acid (MPC-*n*BMA-*N*VA, Table.1) and purified by the dialysis. Poly(ethylene grycol) that has succinimide group and maleimide group was reacted with the copolymer to introduce the maleimide group. The copolymer was dissolved in ethanol and coated on slide glass surface that was cleaned by ozone. The coating was evaluated by X-ray photoelectron spectroscopy and water contact angle measurement. The micro-chamber that has a vertical crossed flow channel was treated with the copolymer and anti-CD34 antibody was immobilized. The suspension of HL60 (CD34 negative) or KG-1a (CD34 positive) was injected and observed by high-speed camera. To increase the interaction of cells on the interface, the micro-chamber was remained to settle out cells on channel surface.

Table.1 Synthesized copolymers.

Sample	MPC:*n*BMA:*N*VA (mol)
NVA 1.0%	30:59:1
NVA 0.5%	30:59.5:0.5
NVA 0.1%	30:59.9:0.1

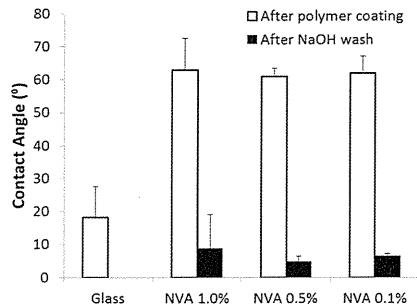

Figure.2 Contact angle analysis of the courted grass surface with MPC-*n*BMA-*N*VA

[RESULTS AND CONCLUSIONS]

The molecular weight of synthesized copolymer (Table.1) was determined by ^{1}H-NMR and GPC. As the result of XPS measurement, the ratio of nitrogen and phosphorus on slide glass surface was increased after the coating. The water contact angle of glass after ozone cleaning was 20°, and the angle was increased to 70° after the polymer coating. When the copolymer coated glass surface was washed with 2.0 N NaOH.aq, the angle was decreased to 10°(Figure2), indicating that the copolymer layer was formed on the glass surface.

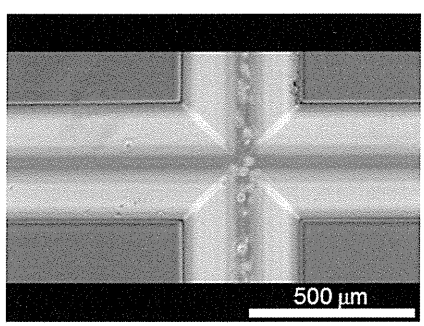

Figure.3 Cross-type micro channel (Volume of cross aria: 12nL、volume of micro channel: 2.9μL)

After the injection of cell suspension into antiCD34 antibody-immobilized micro-chamber, the non-specific adhesion was not observed. The amphiphilic copolymer coating was easy method to modify the glass surface and effectively inhibit the non-specific adhesion.

[REFERENCE]

[1] Mahara A and Yamaoka T, Continuous separation of cells of high osteoblastic differentiation potential from mesenchymal stem cells on an antibody-immobilized column. Biomaterials, 2010, 31: 4231-4237.

[2] Kitano H, Suzuki H, Matsuura K and Ohno K, Molecular Recognition at the exterior surface of a zwitterionic telomere brush. Langmuir, 2010, 26: 6767-6774.