別添５

研究成果の刊行に関する一覧表レイアウト

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
該当なし							

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Mahara,A., Chen H., Ishihara K., Yamaoka T	Phospholipid polymer-based antibody immobilization for cell-rolling surface in a system for stem cell purification.	Journal of Biomaterial Science: Polymer Edition	Under revise process		2014
Mahara, A., Chen H., Carlos A., Ishihara K., Yamaoka T.,	Vertical crossed micro-chamber for cell rolling column to lead definite cell rolling	Preparation			

学会発表

演者	演題名	学会名	場所	開催年月日
馬原　淳	細胞ローリングを利用した細胞診断用マイクロチップの開発	文部科学省・科学研究費補助金・新学術領域研究「ソフトインターフェースの分子科学」	東京	２０１３年7月１１日-１２日
Atsushi MAHARA	Cell-rolling microchip for the detection of circulating -tumor cells	ACS fall meeting	Indianapolis, USA	２０１３年９月８日ー１２日
Atsushi MAHARA	Circulating tumor cell detection system on cell rolling microchip	The 15th International conference on biomedical engineering	Singapore	２０１３年１２月４日-７日
Atsushi MAHARA	Cell-rolling microchip for diagnosis of peripheral circulating cells such as CTCs	ACS Spring meeting	Dallas, USA	２０１４年３月１６日-２０日

IV. 研究成果の刊行物・別刷

Cell-rolling microchip for the detection of circulating-tumor cells

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INTRODUCTION

Circulating tumor cells (CTCs) are existing in the peripheral blood of cancer patient, and relation of CTCs to the metastatic spread of carcinomas has been generally recognized in not only basic research field but also the clinical stage[1]. However, a few CTCs are circulating in the blood flow. Recent study focused on the CTCs detection and characterization by using the microchip technology[2-4]. Antibody-immobilized chip and filter system which could capture the specific cells on the microdevice has been widely investigated for the detection of the rare CTCs. However, non-specific absorption of the other cells has not been suppressed in the detection of CTCs, and its sensitivity is greatly reduced. Here, we have developed the cell-rolling microchip for the specific detection of various cells. In our previous work, the cell-rolling column was developed for the separation of stem cells. This column separated the specific cells by the cell rolling velocity under the media flow. In this study, this mechanisms was applied for the cell detection on the microchip systems. The detection sensitivity would be increased by the evaluation of rolling-velocity because of the continuous interaction between the cell surface and immobilized ligand. To immobilize the antibody, copolymers of poly[2-methacryloyloxyethyl phospho-rylcholine (MPC)-*co*-*n*-butyl methacrylate (*n*BMA)-*co*-*N*-vinylformamide (*N*VF)] (PMBV) were synthesized by random polymerization. Microflow pass (width: 300μm, depth: 100μm) were coated with the polymers, and atni-CD34 antibody were covalently attached by the crosslinker. Cell-rolling velocity of cultured cells on the microchip system was evaluated.

EXPERIMENTAL

Polymer synthesis and preparation of microchip Amphiphilic phospholipid polyjmer (PMBV) with MPC, nBMA and NVF was synthesized by radial polymerization of corresponding monomers using α,α'-azobiisobutyronitrile (AIBN) as an initiator. The monomers and initiator were dissolved in ethanol, and the mixture was stirred at 60˚C for 6h. After the reaction, the polymers were precipitated twice by hexane and diethyl ether. Composition and molecular weight of the two polymers were determined by 1H-NMR (Geminn 2000/300; Varian Inc., CA, USA), and GPC (Shodex SB804-HQ; Showa Denko K.K., Tokyo, JAPAN) in mixed solvent (EtOH:Pure water =0.7:0.3, included 10mM LiBr), respectively. The MPC/nBMA/NVF composition of PMBV40 and PMBV30 were 0.1/0.5/0.1, and 0.3/0.6/0.1, respectively. To produce the amino group in the polymer, the polymers were hydrolyzed with 60mL of 2N HCl. After the neutralization, the polymers were purified with the dialysis tube (Figure 1).

Microchip for the detection of cell-rolling velocity was manufactured by Institute of Microchemical Technology Co., Ltd., (Kanagawa, JAPAN). The microchip channel pattern was specifically designed for the evaluation of cell-rolling velocity (Figure 2). Microchip channel was coated with the copolymers, and the amino groups of the polymers were activated with the NHS-PEG-Maleimide crosslinker (Thermo Scientific, Hudson, NH, USA). Reduced antiCD34 antibody was added into the channel and then the antibody was covalently conjugated on the channel through the activated copolymers.

Evaluation of cell-rolling velocity on the microchip The microchip was connected to the Microfluidics Flow Control System (MFCS; Fluigent, Paris, FRANCE) to strictly control the media flow in the channel. The KG-1a and HL-60 cells were used as the model cells that cells were CD34 positive and negative cells, respectively. Cell suspension was circulated in the sample channel, and the cells were injected into the detection channel by the change of media flow direction. Rolling velocity were monitored on the CCD camera, and the velocity were analyzed on the personal computer.

RESULTS and DISCUSSION

Molecular weight (Mn) and the molecular weight distribution (Mw/Mn) of the synthesized polymer were about 3-6 × 10^{-4} and 1.1-1.3, respectively. When the glass chip was incubated with the polymer solution, the contact angle was largely changed, and the polymer-derived phosphate and nitrogen signal were observed on the X-ray photoelectron spectroscopy. These results indicated that the polymers were stably absorbed on the grass surface by the courting. The conjugation of the

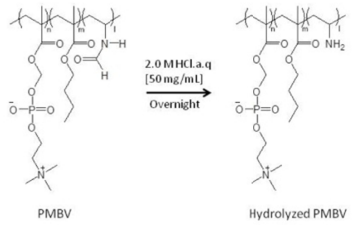

Figure 1 Structure of PMBV and hydrolyzation.

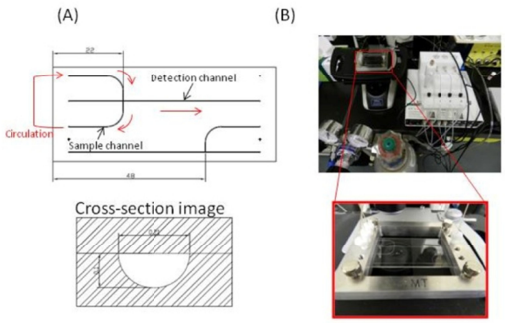

Figure 2 (A) Design of microchip for CTCs detection and (B) the chip and media-flow control system.

antibody could be traced by the radio-isotope experiments. The glass-made microchip channel was courted by this protocol, and the cell-rolling velocity was evaluated.

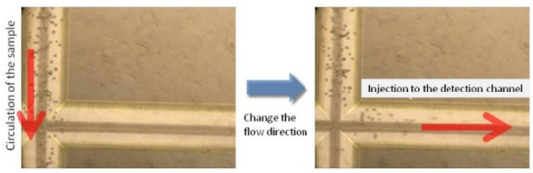

Figure 3 Detection system of cell-rolling velocity on the CTCs microchip channel.

KG-1a and HL-60 cells were used as CD34 positive and negative cells for the model system of CTCs detection, respectively. After the injection of the cell suspension, and the cells were stably circulated by the media flow without any non-specific absorption on the surface (Figure 3). When we used the optimized condition at 0.4 μl/min of media flow, the rolling velocity of KG-1a (CD34 positive) cells on the chip system was detected as about 40 μm/sec. On the other hands, the velocity of the negative cells were about 55 μm/sec. We observed

the significant difference between these rolling velocities. When the media flow was increased, the significant difference of the rolling-velocity was not indicated, and the cell-moving speed was almost same. Therefore, the significant difference was derived from the specific interaction of the surface maker and immobilized ligand.

CONCLUSION

Here, we successfully discriminate the cell type on the microchip by the cell-rolling velocity without any non-specific absorption. This chip system would apply for the CTCs detection system based on the cell-rolling mechanisms.

ACKNOWLEDGEMENTS

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