Development of novel bioanalytical system based on QCM for single cell/materials interactions

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Statement of Purpose: In the recent tissue engineering, the importance of controlling cellular behavior and material-cell interaction is increasing more and more. For example, hybrid artificial organs attracted in the regenerative medicine are new ideas to aim to construct the functional actual organs by using the function of differentiated cells and building the cell into the polymer artificially. To make cells built in live in the states similar to in vivo, materials used in this field should be designed finely by considering material-cell interaction. The study for understanding the material-cell interaction is cellomics research. Cellomics research, which understands reversible and irreversible mechanism of complex network of the cell by focusing on a single cellular behavior on the materials, is widely studied in the biology field. A material used in these fields should be designed precisely to prevent the unexpected biological reaction.

In this study, the clarification of cellular behavior, especially the mechanism of single cell interaction on the materials surface, is focused on by using novel bioanalytical system based on high sensitive quartz crystal microbalance (QCM) and functionalized microchannel chip.

Methods: The conceptual scheme of QCM system for the analysis of cellular behavior on the material surface is shown in Fig.1. Microchannel chip was made of polydimethylsiloxane (PDMS), and the channel surface was modified with previously reported phospholipids(PC) polymer[1] which was inspired from the cell membrane structure to inhibit the non-specific cell adhesion. To examine a single cell separation, we took fluorescently-stained L929 cell suspension (medium: Dulbecco's Modified Eagle Medium) into the inlet of the microchannel by controlling the density of cells and flow velocity. The cellular behaviors [2500, 250, 25 cells/50μL (capacity of QCM chamber) L929 cell suspension] on the materials at 37 ℃ are summarized from the data of the frequency change (Δf) and resistance change (ΔD), and the cell geometry as a function of cell culture time was observed by microscope. In this study, we used 2 kinds of materials; Au, and poly(2-methacryloyloxyethyl phosphorylcholine (MPC) –*co*–2-(methacryloyloxy) ethylthiol)(PMSH) having a thiol group in the side chain which binds covalently to Au surface.

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Fig.1 Schematic illustration of QCM system.

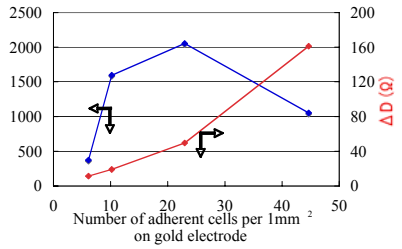

Fig.2 Correlation between Δf, ΔD values and the number of cells attached.

Results/Discussion: By using the microchannel chip (Fig.1), a desired number of L929 cell was taken by controlling the density of 30000 cells/mL and flow velocity of 0.10 μL/min. Therefore, we have examined interaction between various materials and desired number cells by the QCM system with the chip.

The typical cellular behaviors on the Au electrode of QCM are shown in Fig.2 from the data of the Δf and ΔD values. The correlation between ΔD and the number of cells attached on the surface suggested that the behavior of single cell attachment and spreading on different materials could measure by the QCM system using ΔD. While the Δf was independent to the number and geometry of cells, for Δf was caused by the difference of the sensitivity of the area on the Au electrode[2], and it was found that the cell attachment area should be limited when analyzing of a single cell behavior with Δf. The problem will solve by making a mask on the periphery of Au electrode using newly designed PC polymer PMSH. The Δf values on PMSH/Au after 3 hours decreases by 80% compared to ones on Au. This indicated the difference of surface structure and PMSH can be used for the non-specific cell adhesion material. From above, the behavior of single cellular behavior could be measured by the QCM system using ΔD, and by controlling attachment area with PMSH, the behavior could be measured by the QCM system using Δf.

Conclusions: We developed QCM biosensor combined with cell separation microchannel for analysis of cellular behavior on materials. By using this system, it is confirmed that the ΔD values have a good relation to the number of attached cells, while Δf values are dependent of attachment area. Such analysis of behavior on materials is a novel method to examine material-cell interaction quantitatively. This system provides us to examine material-cell interaction and design materials for tissue engineering.

References: [1] J. Sibarani et al. Coll. and Surf. B: Biointerface, 2006, in press [2] C. Kurosawa et al. Proceedings of the 2004 IEEE International Frequency Control Symposium and Exposition,(2005.3), 554-557