Small Change of Environmental pH Within Near-neutral Range Will Dramatically Change the Cell Adhesiveness on Chitosan Surface

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Statement of Purpose: To culture mammalian cells, the suitable initial environmental pH of media is considered as 6.9 to 8.5 on tissue culture plates, depending on the species and cell type. As the culture substrate is, however, an environmental pH sensitive polyelectrolyte, the effect of environmental pH is not only to cells but also to the substrate. Therefore, the cell-substrate interaction must be changed. Chitosan is a nature polymer composed of two kinds of monomer, glucoseamine and acetyl-glucoseamine. Because of the primary amine on glucoseamine, its physicochemical characteristic made chitosan a good candidate to be applied to the pH-responsive approach in biomedical field. In our best knowledge, however, the effect of environmental pH to the cell adhesiveness on chitosan within the near-neutral range has never been discussed. In this study, we were trying to discuss the effect of environmental pH to the relationship and its possibility to application.

Methods: The chitosan-coated surface was made from 1% chitosan (deacetylation degree =80%, pKa=6.50) in 3% acetic acid solution by dry casting process. The equilibrium of sodium bicarbonate and CO_2 pressure was used to create the different pH environment within near-neutral range. Six pH DME media (Invitrogen, MO) with 10% fetal bovine serum (Invitrogen, MO) was prepared via adding different concentration of sodium bicarbonate from 600mg/mL to 3600mg/mL. All media pH were adjusted to 7.40 before sterize entered the incubator with 5% CO_2 at 37°C. To measure the equilibrium pH of media in the 5%CO_2, 1ml of six media was added to 24-well and incubated 1hrs and 24hrs. The adsorption of plasma fibronectin on chitosan-coated surface in these six pH condition was quantified by Western Blot. Four cell types, including HaCaT (human keratinocytes cell line), H1299 (human lung carcinoma cell line), 3T3 (mouse embryonic cell line), and bovine corneal fibroblast were seeded to the chitosan-coated 24 well in these six media. The cell adhesiveness was then measured by CyQuant® Assay (Invitrogen, MO). Here, the cell adhesiveness was defined as the ratio of cell adhered on substrate to the initial seeded number. As control, the cell adhesiveness on TCPS was also examined.

Results: After incubation for 1hrs with 5%CO_2 in 37℃, the pH of media was 6.99 (0.02), 7.20 (0.01), 7.36 (0.02), 7.48 (0.03), 7.57 (0.02), 7.65 (0.01) as the concentration of sodium bicarbonate was 600mg/ml, 1200mg/ml, 1800mg/ml, 2400mg/ml, 3000mg/ml, and 3600mg/ml, respectively. The pH value had no significant difference when keeping media in the incubator for 24hrs. According to Western blot, the plasma fibronectin adsorbed on chitosan-coated surface was decreased when the media pH was raised from 6.99 to 7.65 (Figure 1). Furthermore, the ratio of cell adhered on chitosan has same trend with the amount of fibronection adsorption. For all cell types, the highest and lowest cell adhesiveness on chitosan was occurred when the media pH was near 6.99 to 7.20 and near 7.65, respectively (Figure 2). The same phenomenon can be observed in phase contrast image. Cell, as shown in Figure 3, adhered on chitosan when media pH was lower than 7.4 but suspended when it was higher than 7.4. We suppose that it have a critical pH for cell adhesion on chitosan within near-neutral range. This phenomon, however, was not observed on TCPS (figure 3).

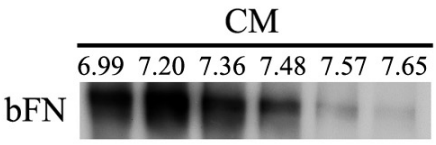

Figure 1. Western blot of plasma fibronectin on chitosan

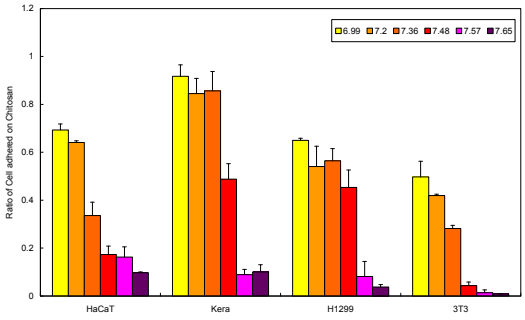

Figure 2. Cell adhesiveness on chitosan of HaCaT, Keratocytes, H1299, and 3T3

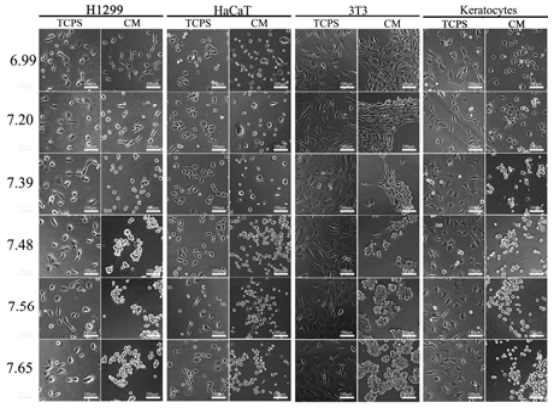

Figure 3. Cell morphology of HaCaT, Keratocytes, H1299, and 3T3 on chitosan and TCPS

Conclusions: Small change of environmental pH within near-neutral range will dramatically change the cell adhesiveness on chitosan surface.