Effect of pK_a of Polymer Microcarriers on Growth of Mouse L Cell

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In opder to obtain fundamental information about the electrostatic effect of microcarriers on fibroblastic cell growth, the aminated poly(γ-methyl L-glutamate) and the cross-linked poly(ε-lysine) beads were prepared. When the growth of mouse L cell on the various beads were determined by common cell culture methods, it strongly depends on the apparent pK_a of the beads but not on their amino-group content. The polymer beads with neutral apparent pK_a (7.4–7.6), just as in the physiological environment, were found to be excellent microcarriers, showing the highest growth of fibroblastic cells.

Recently various mammalian cells have been used for producing recombinant DNA products.[1] For large-scale proliferation of anchorage-dependent mammalian cells, such as fibroblast, microcarrier system[2,3] is proven to be the most effective. M. Kiremitci et al.[4] described previously that introduction of amino groups into the microcarriers significantly increased the amount of attached cells, but it caused toxicity in the culture media when it was used in large amounts.

In this study, we describe the effects of amino-group content of microcarriers and their apparent pK_a (p$K_{a,app}$) on growth of fibroblastic cells to obtain detailed information about the most suitable surface environment of microcarriers. Aminated poly(γ-methyl L-glutamate) (PMLG-NH_2) beads, cross-linked poly(ε-lysine) (PL) beads, Cytodex 1 (DEAE-Sephadex) (Pharmacia), and polyallylamine beads (Nitto Boseki Co. Ltd.) were used as miarocarriers. PMLG-NH_2 beads were prepared by the suspension–evaporation method[5,6] with PMLG (1 unit: –NH–CH$(CH_2)_2$COOCH_3–CO–, degree of polymerization: 1000, Ajinomoto Co. Ltd.), and then amination[7] as described previously. PL beads were prepared by suspension copolymerization (in a paraffin liquid) with poly(ε-lysine) (originating from *Streptomyces albulus*, 1 unit: –NH–$(CH_2)_4$CH(NH_2)CO–, degree of polymerization: 25, pK_a: 7.6, Chisso Co. Ltd.)[8] and chloromethyloxirane. The amount of amino groups of PL and PMLG-NH_2 beads were adjusted by controlling the ratio of poly(ε-lysine) (in cross-linking) and the amination condition (time and ratio of diaminoethane into the beads), respectively. The beads obtained with diameters of 100 to 300 were used as microcarriers. The amino-group content and the p$K_{a,app}$ of the beads were quantified by pH titration.[7]

It has already been found[9] that the p$K_{a,app}$ of PL beads originating from poly(ε-lysine) is lower than that of PMLG-NH_2 beads originating from poly(α-amino) acid. From this favorable different property, we can prepare various cationic polymer beads, which have a different range of p$K_{a,app}$, although they have a content similar to that of amino groups.

As shown in Figure 1, The p$K_{a,app}$ of PL increased from 5.0 to 7.0, while its amino-group content increased from 1.3 to 4.6 meq g^{-1}. As for PMLG-NH_2, it increased from 7.4 to 8.2 with increasing the amino-group content from 0.2 to 3.3 meq g^{-1}. The p$K_{a,app}$ of PL is always lower than that of PMLG-NH_2 in all amino-group content, because carbonyl groups of PL have an electron-accepting effect on its free amino groups easily. In addition, the free amino groups of PL and PMLG-NH_2 are placed at the α-and γ-position in these structures, respectively.

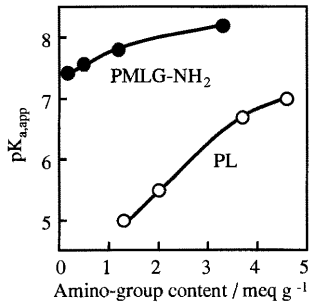

Figure 1. Relationship between amino-group content and apparent pK_a (p$K_{a,app}$) of various beads.

All the cell experiments were performed using fibroblastic mouse L929 cells. The cells were originally obtained from the Chemo-Sero-Therapeutic Research Institute. The cell-growth medium was Dulbecco's modified Eagle's medium (Gibco, USA) with 10% fetal bovine serum (Hyclone, UT), and the cells were routinely maintained by common methods.[10] Growth characteristics of the cells on the beads were studied in the stationary culture conditions as follows: 2.5 mL of wet beads were incubated in 45 × 10 mm glass laboratory dishes at 37 °C for 1 h, then 5 mL of 2×10^4 cells ml^{-1} (1×10^5 cells $dish^{-1}$) was suspended. The cultures were maintained in a 5% CO_2 environment at 37 °C. The cell-growth activity on beads was estimated as total cell counts attached on beads after incubating for 2–6 days (S_t) per initial cell count (S_0).

The effect of amino-group content of beads on cell growth was extremely variable. Cells attached to beads were over 80% of S_0 in all beads after incubating for 120 min. In the case of PMLG-NH_2, the smaller was the content of amino groups, the higher was the cell growth; with increasing the culture time from 2 to 6 days, the beads with amino-group content of 0.5 meq g^{-1} (NH_2-0.5) increased the cell growth from 1.10 to 19.10 $S_t\ S_0^{-1}$ but the NH_2-3.3 decreased from 0.80 to 0.30 $S_t\ S_0^{-1}$ (Figure 2a). By contrast, in the case of PL, the larger was the amino-group content, the higher was the cell growth; the NH_2-3.7 increased the cell growth from 0.88 to 14.50 $S_t\ S_0^{-1}$ but the NH_2-1.3 decreased from 0.35 to 0.15 $S_t\ S_0^{-1}$ (Figure 2b).

Figures 3a and b show the effects of amino-group content of various beads and their p$K_{a,app}$ on cell growth after incubat-

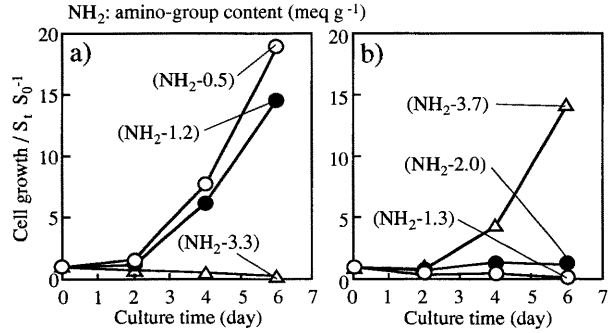

Figure 2. Effect of culture time on cell growth by (a) PMLG-NH_2 and (b) PL beads. The cell growth was determined by a stationary culture method with 2.5 ml of the wet beads and 5 ml of Dulbecco's MEM supplemented with 10% fetal bovine serum. S_t: total cell count on beads after incubating for 2-6 days; S_0: initial cell count.

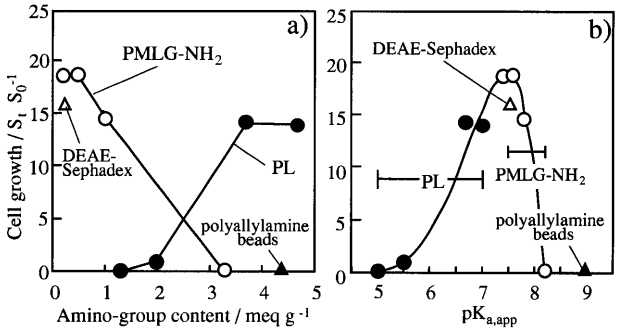

Figure 3. Effects of (a) amino-group content and (b) $pK_{a,app}$ on cell growth by various microcarriers. S_t: total cell count on microcarriers after incubating for 6 days; S_0: initial cell count.

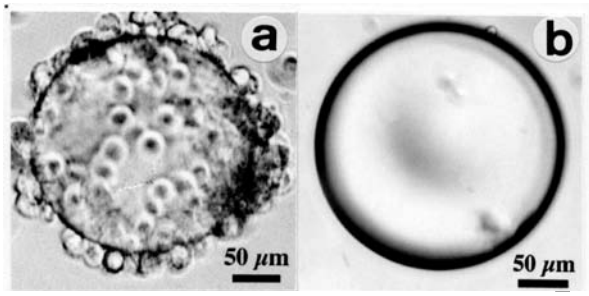

Figure 4. Optical micrographs of mouse L cells on PL microcarriers with (a) pKa,app: 7.0 and (b) pKa,app: 5.0 after incubating for 6 days.

ing for 6 days, respectively. The results showed that the cell growth was strongly dependent on the $pK_{a,app}$ but not necessarily on the amino-group content. As shown in Figures 4a and b, the PL ($pK_{a,app}$ 7.0) showed high cell growth but the PL ($pK_{a,app}$ 5.0) showed extreamely low growth. As shown in Figure 3b, the beads with $pK_{a,app}$ 7.4–7.6 showed the highest cell growth in all of beads. This founding was supported by the experiment with other commercially available aminated beads; the DEAE-Sephadex ($pK_{a,app}$ 7.5) showed high cell growth but, in the case of polyallylamine beads ($pK_{a,app}$ 8.9), no cell growth was seen.

Ishida et al.[11] described previously that when the cell growths of fibroblasts (mouse L) on the poly(α-amino acid)s-coated dishes were estimated, a moderate amount of the net charges on poly(α-amino acid)s were important for cell growth. It has also been reported[4] that the microcarriers with large amount of amino groups caused toxicity in the culture media. However, in the case of PL beads with a large amount of amino groups (over 3.7 meq g^{-1}), the cell showed high growth (Figure 3a). The cell growth increased when the $pK_{a,app}$ value of beads approached a neutral one (Figure 3b), which is the most suitable environment for cell culture. Kunitake et al.[12] found that ionic compounds act as a buffer at their pK_a. The result (Figure 3b) suggests that similar buffer effect can be observed only at local area near surface, even if ionic units being immobilized on beads. Therefore, we consider that local pH conditions on surfaces, where cells attach, are controlled by $pK_{a,app}$, and acidic or basic micro-environments on the surfaces prepared by beads with acidic or basic $pK_{a,app}$, are detrimental for living and growing of cells (see graphical abstract).

In conclusion, we found that the environment for cell growth is controlled by microcarrier's $pK_{a,app}$ but not necessarily by their amino-group content. In this study, the polymer beads with $pK_{a,app}$ 7.4–7.6 indicate the most suitable surface-pH environment for increasing attached cell growth significantly.

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