Osteogenic Differentiation of Human Mesenchymal Stem Cells Cultured on Grafted Polyelectrolyte Surfaces
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Introduction
The effects of surface properties such as chemical composition, roughness, and surface charge on stem cell functions will provide important information on the design of biomaterials for tissue engineering. However, information on the effect of chemical composition, especially surface charge, on stem cells function is very limited. In the present study, we used a photochemical method1 to introduce positively charged polyallylamine (PAAm) and negatively charged poly(acrylic acid) (PAAc) on cell-culture polystyrene plate surfaces and investigated their effects on the osteogenic differentiation of human mesenchymal stem cells (MSCs).

![Chemical structures of (a) polyallylamine (PAAm) and (b) poly(acrylic acid) (PAAc).](image)

Fig. 1. Chemical structures of (a) polyallylamine (PAAm) and (b) poly(acrylic acid) (PAAc).

Materials and Methods
Photoreactive azidophenyl-derivatized PAAm was synthesized by coupling PAAm with N-(4-azidobenzoxyloxy) succinimide. Azidophenyl-derivatized PAAc conjugate was synthesized by coupling PAAc with 4-azidoaniline. The aqueous solutions of the obtained polymers were placed in the wells of cell-culture polystyrene plates and air dried. The plates were then irradiated with ultraviolet light. Human MSCs at passage 4 in DMEM serum medium were seeded onto each well of the PAAm-grafted, PAAc-grafted, and non-grafted wells in the plates. To induce osteogenic differentiation, the cells were cultured in DMEM supplemented with dexamethasone (DEX) and β-glycerophosphate disodium salt for 3 weeks. Osteogenic differentiation was assessed by alkaline phosphatase (ALP) staining, alizarin red S staining, and gene expression analysis using real-time RT-PCR.

Results
After culture passage 4, the MSCs were cultured on the grafted surfaces in osteogenic induction and control media. The MSCs adhered, spread, and proliferated more easily on the PAAm-grafted surface than they did on the PAAm and polystyrene surfaces in the control medium. MSCs cultured on the surfaces were positively stained with ALP and alizarin red S in the presence of DEX, while the cells cultured without DEX were not. Real-time RT-PCR results indicated that MSCs cultured on these surfaces in the presence of DEX expressed osteogenic marker genes encoding ALP, osteocalcin, bone sialoprotein, osteopontin, and type I collagen.

Discussion and Conclusions
PAAm- and PAAc- grafted and non-grafted cell culture polystyrene surfaces supported the osteogenic differentiation of human MSCs in the presence of dexamethasone but not in the absence of dexamethasone. The differentiation required the synergistic effect of dexamethasone. These results will be useful for the design of biomaterials used for tissue engineering.

References

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