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Mini Review

Keywords
Biosilane,
Cell affinity,
Surface modification

Effects of Bone Formation deriving biosilanes

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Abstract

The aim of this study was to construct the material surface that allows cells to be compatible with it without their destruction by silane coupling agents having amide group (biosilanes). These biosilanes were expected to make a soft landing to cytoplasm through the hydrogen bonding between their amide groups and cells. Evaluations of cell affinity using glass substrates modified with the synthesized biosilanes revealed that many cells remain on the modified glass plate. In addition, the implantation into the body of immunodeficient mouse of a composite material composed of porous hydroxyapatite and osteoblast showed the formation of a bone-like structure.

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The objective of this research is to describe the synthesis of highly biocompatible and bone formative surface modifying agents for the development of materials used in medical treatment and the examination of cell affinity with the material surface modified with the compounds synthesized[1]. The six surface modifying agents synthesized are amide group containing silane coupling agents (biosilanes).

Fig. 1 showed the chemical structures and the codes of the biosilanes synthesized in this study. The micro-cover glass plates modified with each of the biosilanes and poly-l-lysine modified cover glass plates as positive control were set in 24-well plates, washed once with ethanol and five times with PBS, and dried[2]. The dispersion of KUSA cells was placed in each of the 24-well plates, and then the cells were incubated for 1 hour at 37°C. Then, the luciferase assay reagents were added to each of the well plates and the luminescence from each well plate was detected with the chemiluminescence detector. β-TCP/osteoblast composite (with β-TCP modified by 1,1-DAU6M) together with unmodified β-TCP as control were subcutaneously implanted into the back skin of an immunodeficient mouse and they were taken out 8 weeks later. The implanted samples taken out of the mouse were immersed in 4% paraformaldehyde solution to fix them. The fixed samples were washed by water and the water in the sample tissues was replaced by ethanol and toluene after being decalcificated in 10% formic acid solution. The samples were immersed in melted paraffin to allow the liquid to penetrate into them. Then, the samples embedded in paraffin were sliced into 3μm thick layers with a rotary microtome for histological observation and each layer was dyed with hematoxylin–eosin to be observed under the optical microscope.

Fig. 2 shows the results of luciferase assay. The luminescence is caused by adenosine triphosphate in the cells and its intensity is proportional to cell number[3,4]. Luminescence intensity for the biosilane-modified glass substrates was higher than that for the

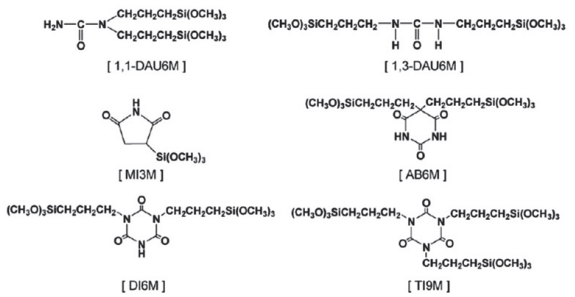

Fig. 1 The chemical structures and the codes of the biosilanes

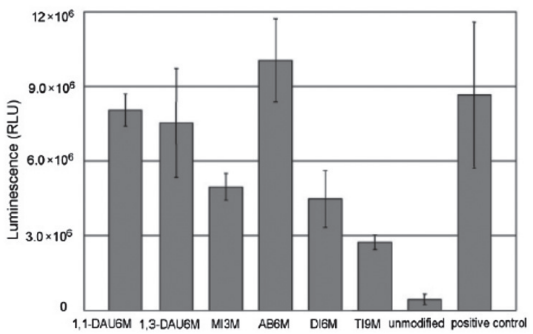

Fig. 2 Relative luminescense intensities of cells absorbed onto glass surfaces

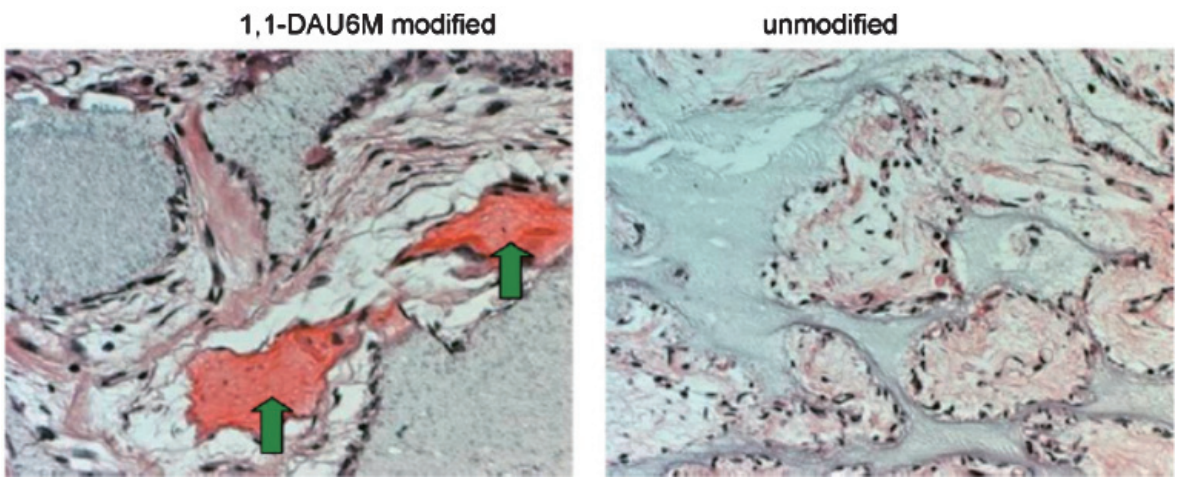

Fig. 3 Hematoxylin-eosin stained images of 1,1-DAU6M modified (two arrows) and unmodified β-TCP/cell composite materials

unmodified substrate, especially the substrates modified with 1,1-DAU6M, 1,3-DAU6M, and AB6M showed an intensity as high as that for poly-l-lysine-modified glass substrate. It was shown that the image of an implanted piece stained with hematoxylin–eosin obtained in the implantation experiment using mouse and β-TCP/osteoblast composite (Fig. 3). While a bone-like structure was observed in the interior of this composite, no such structure was found for unmodified β-TCP. This would be interpreted as indicating that osteoblasts are hardly fixed on unmodified β-TCP, whereas the cells easily attach to the modified β-TCP through the interaction of amide groups of biosilane on its surface with the cells, thereby making the cells possible to wander into the interior of β-TCP.

It was suggested that the biosilanes prepared in this work are expected to greatly contribute to the future bone-regenerating medical treatment.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Gary E. D., Su P., Deron W. and Hiroshi M. (2001). Fabrication of Protein Tubules: Immobilization of Proteins on Peptide Tubules. *J. Phys. Chem. B.* **105**: 7612-7618.
2. Perla F., Gabriella R., Antonella F., et al. (2001). Modulation of osteosarcoma cell growth and differentiation by silane-modified surfaces. *J. Biomed. Mater. Res.* **55**: 338-349.
3. Klemke R. L., Yebra M., Bayna E. M. and Cheresh D. A. (1994). Receptor tyrosine kinase signaling required for integrin alpha v beta 5-directed cell motility but not adhesion on vitronectin. *J. Cell Biol.* **127**: 859-866.
4. Anne L. P., Gerald M. E., and Kathryn L. C. (1993). Multiple integrins mediate cell attachment to cytotactin/tenascin. *Proc. Natl. Acad. Sci. USA*. **90**: 10154-10158.