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Research Article

Application of Imogolite, Almino-Silicate Nanotube, as Scaffold for the Mineralization of Osteoblasts

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Abstract Imogolite is a naturally occurring alumino-silicate clay mineral with a nanotube structure. In this study, synthetic imogolite nanotubes were applied to cell culture and the properties as scaffold were compared with those of a conventional culture dish and the carbon nanotube scaffold. The surface characteristics of the imogolite scaffold were drastically changed with the amount of imogolite on the dish. Human osteoblast-like cells (SaOS2) on imogolite scaffolds showed a widely spreading morphology and a high functional expression. Particularly, the scaffold with the large amount of imogolite provided the suitable environments of topography, roughness, wettability and protein adsorption ability for widely spreading morphology and differentiation of osteoblasts. Imogolite retains various positive factors as scaffolds for osteoblastic proliferation and differentiation and is expected for the application to bone tissue engineering.

Keywords nanotubes; imogolite; osteoblast; scaffold; mineralization

1 Introduction

Imogolite is a naturally occurring white hydrous aluminum silicate clay mineral with a typical composition of $[Al_2O_3\text{-}SiO_2\text{-}2H_2O]_n$. This material has a unique tubular structure of about 2 nm diameter and 60 nm–2 μm length (Figure 1), which is similar to the structure of single walled carbon nanotubes (SWCNTs). We have reported that imogolite applied to scaffolds for cell culture and then osteoblast-like cells on those scaffolds showed the good proliferation and the adhesion. In this study, osteoblastic functions were investigated by measuring of mineralization in osteoblast-like cells (SaOS2) on imogolite scaffold, compared to a conventional culture dish and the carbon nanotubes scaffold.

2 Materials and methods

2.1 Specimen

The specimen was provided as a white slurry (1 mg/mL) from National Institute of Advanced Industrial Science and Technology, AIST (Institute for Geo-Resources and Environment, Tsukuba, Japan) that contained synthetic imogolite nanotubes of about 2 nm diameter and 60 nm–2 μm length. Single walled carbon nanotubes (SWCNTs) synthesized by the arc discharge method were used for comparison (Meijo Nano Carbon Co., Nagoya, Japan). The purity was 90% and the size was 0.9–1.5 nm diameter and 2–3 μm length. They were purified with hydrochloric acid to remove the impurities of metal catalysts.

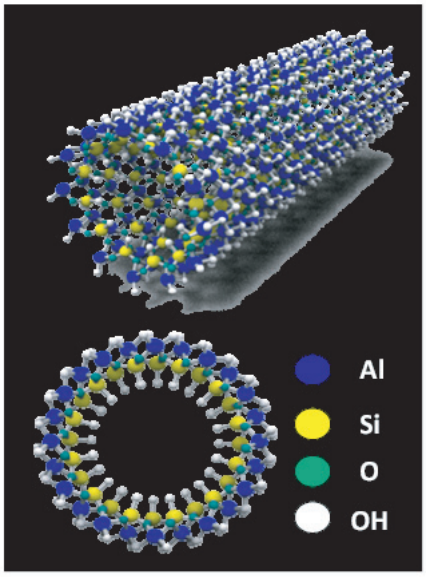

Figure 1: Schematic structure of imogolite.

2.2 Preparation of scaffolds

Imogolite slurry was diluted by deionized water to concentrations of 0.01 mg/mL and 0.1 mg/mL. Then, 2 mL of imogolite slurry was poured into a cell culture dish (CORNING, MA, USA) of 60 mm diameter. Imogolite was fixed on the cell culture dish by drying for 7 days at room temperature. A cell culture dish without additives was used as a control (Cntl). To prepare CNT scaffolds, a dilute solution of CNTs in 99.5% ethanol (5 μg/mL) was dispersed by ultrasonication. An aliquot of the CNT dispersion was immediately spotted onto a cell culture dish. They were then dried at room temperature. This procedure was repeated until the necessary amount of 10 μg CNT per dish was obtained. All of the scaffolds were sterilized by ultraviolet radiation for 48 hours. In the following, the scaffolds with imogolite at 20, 200, and 2000 μg/dish and CNTs are abbreviated as Im20, Im200, Im2000 and CNT, respectively.

2.3 Cell culture

1.0×10^4 human osteoblast-like cells (SaOS2) were seeded onto each scaffold (Im20, Im200, Im2000, CNT and Cntl) and cultured in Dulbecco's modified Eagle's medium (DMEM; SIGMA, MO, USA) with 10% fetal bovine serum (FBS; Biowest, FL, USA) and 1% penicillin-streptomycin under standard cell culture conditions (37 °C in a humidified 5% CO_2/95% air environment) for 14 days.

2.4 SEM observation

The morphology of scaffolds was observed using a scanning electron microscope (SEM) (S-4000; HITACHI, Tokyo, Japan).

2.5 Fluorescence microscopic observation

After 3 days of culture, SaOS2 cells were fixed in 10% formalin and stained using fluorescent dyes, rhodamine phalloidin (Millipore, MA, USA). Fluorescence microscopy was used to examine cell morphology and actin filaments.

2.6 Mineralization, calcium assay

Concentrations of Ca ions of cell layers also containing mineralized extra cellular matrix (ECM) were estimated with Calcium C-test (Wako Pure Chemical Industries, Osaka, Japan). Cells on scaffolds after culturing for 7 and 14 days were soaked with 1 M hydrochloric acid, and then the extract solution was used for Ca assay.

2.7 Statistical analysis

All the data are given as the means $\pm$ SD. Statistical differences were analyzed using one-way analysis of variance (ANOVA). Values of $P < .05$ were considered to be statistically significant.

3 Results and discussion

The properties of scaffolds play a pivotal role in controlling the cell growth and morphology and impose a direct influence on intra-cellular response. Cell behavior such as adhesion, spreading and proliferation represents the initial phase of cell-scaffold communication that subsequently affect differentiation and mineralization. Figure 1 shows the schematic structure of imogolite. The tube shell is composed of HO-Si-O-Al-OH for the radial direction from the inner surface of the tube structure to the outer surface. All of the outermost surfaces are covered with -OH, resulting in high hydrophilicity. The nanoscaled diameter and tube structure result in a high surface area and high adsorption capacity. Figure 2 shows SEM images of the surface morphology of scaffolds. Both SWCNTs and imogolite nanotubes have a tendency to agglomerate and form bundles easily. In the case of CNT (Figure 2(a)), string-shaped bundles of about 50 nm diameter were sparsely distributed on the dish. The imogolite scaffolds were composed of bundles of about 20 nm diameter, and their morphology was significantly changed depending on the concentration of dispersed solution. At a lower concentration of imogolite (Im20), an island-like shape was formed in a random orientation and covered almost the whole dish, as shown in Figure 2(b). With further increase of the concentration (Im200), the morphology showed the characteristics of self-organization and the bundles were densely aligned in the same direction. The surface was completely covered (Figure 2(c)). At the highest concentration (Im2000), the imogolite bundles covered the dish in a random orientation and overlapped by several layers vertically (Figure 2(d)). The nano-fibrous scaffolds were developed to mimic the fibrous morphology of type I collagen, a major component of bone ECM known to affect on osteoblastic behavior. In this study, the imogolite bundles were randomly aligned in Im2000

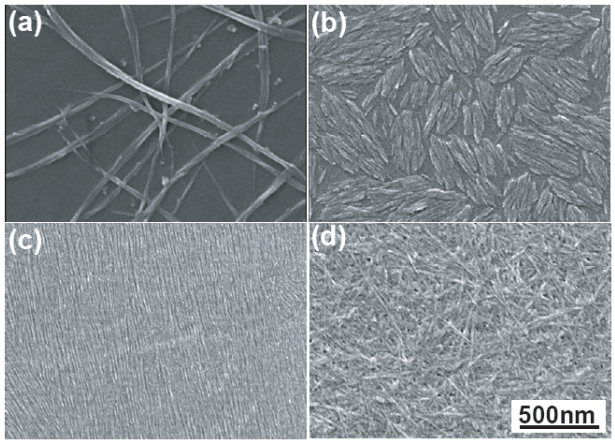

Figure 2: SEM images of scaffolds for (a) CNT, (b) Im20, (c) Im200 and (d) Im200.

(Figure 2(d)), which was similar to that of type I collagen fibrils in ECM. We have reported that Im2000 adsorbed the larger amount of protein compared with Im20 and Im200, because of the higher specific surface area due to the mesoporous structure consisting of random, overlapped imogolite bundles [2]. This property would increase the positive effect on the cell attachment, proliferation and differentiation. Cells on Im2000 spread in all directions and actin filaments also extended for various directions (Figure 3). This result indicates that cytoskeleton developed and the nucleus was clearly extended. On the other hand, those on Cntl elongated in one direction and actin filaments were aligned to parallel the long axes of cells. Aoki et al. reported that human osteoblast-like cells (SaOS2) spread well in all directions and expressed the higher osteoblastic functions on the CNT scaffolds, compared with the macroscopic graphite scaffolds [1]. Watari et al. reported that the adsorption of protein on nano-fibrous scaffolds and its nano-meshwork structure contribute to the excellent cell adhesion and growth [3]. We could speculate that nano-fibrous scaffolds adsorb the larger amount of protein in culture medium due to the higher specific surface area and affinity, resulting in those showing a good biocompatibility. In addition, the geometrical factor of nano-meshwork structure would induce the cell responses to develop the nano-scaled structure in cells, such as filopodia and actin filaments. Then cells were widely spread and strongly bound on nano-fibrous scaffolds. Figure 4 showed that calcium concentrations significantly increase at Im2000 compared with other scaffolds. It is probable that various characteristics of Im2000, physical factors such as surface morphology, roughness, wettability and chemical factors such as released various ions enhanced osteoblastic differentiation. Values of Cntl and Im200 were almost the same. We could speculate that the characteristics of Im2000 for cell culture were random topography, appropriate roughness, high wettability and high protein adsorption capacity. The details were not clear, those of Im2000 might be shown positive effect for cell morphology, proliferation and differentiation in human osteoblast-like cells.

4 Conclusions

In this study, osteoblasts on imogolite scaffolds showed widely spread and high functional expression. In particular, Im2000 provided the suitable environments of topography, roughness wettability and protein adsorption ability for the spread and differentiation of osteoblasts. Imogolite retains various positive factors as scaffolds for osteoblastic proliferation and differentiation and is expected for the application to bone tissue engineering.

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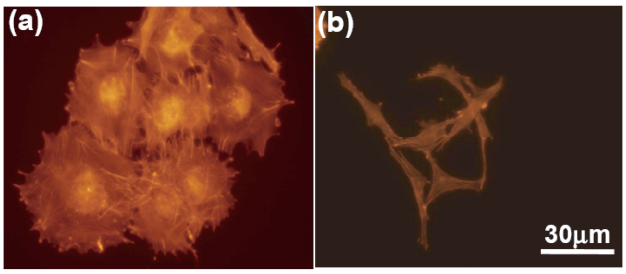

Figure 3: Fluorescence microscopic images stained for actin filaments of cultured SaOS2 cells on (a) Im2000 and (b) Cntl.

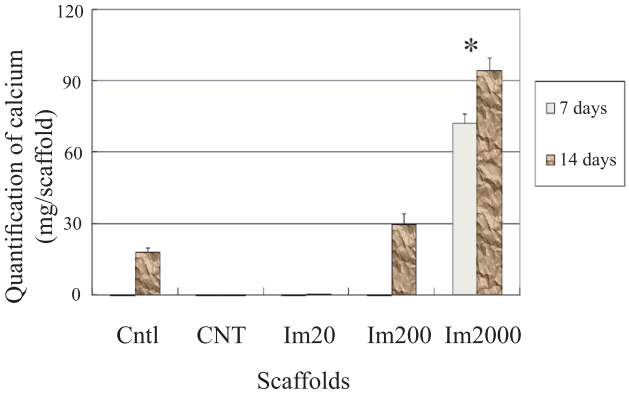

Figure 4: Quantification of mineralization: calcium deposition in SaOS2 cells.

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