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Osteoblastic MC3T3-E1 cells on diamond-like carbon-coated silicon plates: Field emission scanning electron microscopy data

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ABSTRACT

Diamond-like carbon (DLC) is an amorphous form of carbon that contains aspects of both the diamond and graphite structures. It is composed of carbon and hydrogen, and owing to its texture, high mechanical hardness, chemical inertness, and optical transparency, DLC is widely used as a protective coating in the form of a thin film, which is applied to the surfaces of many materials. Recently, it has attracted attention as a biomedical material because of its high biocompatibility and stability [1,2]. DLC is particularly suitable to be embedded in the body owing to its low friction properties and selective cell surface attachment properties [3]. The material is currently being developed for the treatment of bone fractures [4]. However, unlike fibroblasts, the attachment of osteoblasts has not been extensively examined and no morphological data is available on how osteoblastic cells form contacts with the surface of biocompatible DLC-coated materials. Herein, such data were collected by coating DLC on the surface of silicon plates. The attachment of mouse cells to the DLC-coated plates was examined by colorimetric cell prolif-

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eration assay, and morphological observations were made using a field emission scanning electron microscope. Also, the flat cross section of the cell and plate was obtained by the ion milling method.

Specifications Table

Subject	Materials Science
Specific subject area	Surfaces, Coatings and Films, Diamond-Like Carbon
Type of data	Data presented as graphs and pictures.
How data were acquired	Data were collected after the coating of DLC on silicon plates using PED-401 (ANELVA Ltd., Japan) on silicon plate and subsequent culture of the MC3T3-E1 cells (ATCC, USA). Cell proliferation was measured using a microplate reader (680XR, Bio-Rad Japan Laboratory, Japan). The field emission scanning electron microscope (FE-SEM) observations were conducted using Regulus8100 (Hitachi High-Tech., Japan). Ion milling was performed with IM4000Plus (Hitachi High-Tech., Japan).
Data format	Raw, Analyzed
Parameters for data collection	Cells (1×10^4) were seeded on noncoated or DLC-coated silicon plates and cultured in Costar® 6-well cell culture plates with 3 mL of culture medium. After fixation of the cells, specimens were subjected to proliferation assays and FE-SEM analysis. Colorimetric assays were performed to measure proliferation and the optical density was measured at 595-nm wavelength. Milling of the sample was performed at an accelerating voltage of 4.0 kV, discharge voltage of 1.5 kV with mode of C4 $\pm$ 30°.
Description of data collection	Fig. 1. Analyzed data of the proliferation of the two cell lines on noncoated or DLC-coated silicon plates. The cell proliferation was measured by colorimetric assay of two groups: the control group with noncoated plates and the group of samples on DLC-coated plates. NIH3T3 and MC3T3-E1 cells were used and the relative number of cells on each plate was measured on days 3 and 7 post-inoculation. Fig. 2. Raw data set of FE-SEM images of the MC3T3-E1 cells: cells cultured on noncoated (a) or DLC-coated (b) plates. The images were taken after fixation, critical point drying, and osmium coating of the plates. They were taken with 2 kV acceleration voltage, 2560 $\times$ 1920 picture element, and a magnification of 1300 or 1,500, respectively. Fig. 3. Raw data set of the flat cross section of MC3T3-E1 cells on noncoated silicon plates. The same sample from Fig. 2 (a) was milled and the cross section was observed using FE-SEM. The images were taken with 800 V acceleration voltage, 1280 $\times$ 960 picture element, and a magnification of 10,000 (a and d) or 70,000 (b, c, and e). Fig. 4. Raw data set of the flat cross section of MC3T3-E1 cells on DLC-coated silicon plates. The same sample from Fig. 2 (b) was milled and the cross section was observed using FE-SEM. The images were taken with 800 V acceleration voltage, 1280 $\times$ 960 picture element, and magnifications of 10,000 (a), 30,000 (d), or 70,000 (b, c, and e). The original images of each figure are also provided in the Mendeley Data repository.
Data source location	Institution: Core Research Facilities, The Jikei University School of Medicine, Tokyo, 105-8461, Japan.
Data accessibility	Mendeley Data Repository Manome, Yoshinobu; Tachibana, Toshiaki; Hiratsuka, Masanori; Sato, Keisuke; Ohgoe, Yasuharu; Hirakuri, Kenji (2021), "Attachment of Osteoblastic MC3T3-E1 Cells on Diamond-Like Carbon-Coated Silicon Plates", Mendeley Data, https://data.mendeley.com/datasets/yt52fw55wx

Value of the Data

- These data is important because uncovering the mode of adhesion of cells on DLC surfaces including the length and figure of filopodia and lamellipodia as well as the coating thickness is of practical use for designing the materials. As a biomaterial, the main issues to consider for surface processing are biocompatibility and the stability of the coating layer. Since attachment surface constantly changes over the time with the movements of the cells and coated material itself is erodible, the data provides information that needs countermeasures for making the film.
- This data can benefit those who want to use DLC especially as an orthopedic application. Since these osteoblastic cells can be easily induced to calcifications *in vitro* by osteogenic reagents [5], it is relatively easy to obtain information on the bone inducibility, production of related cytokines, effects of neighboring cells as well as gene expressions of osteoblastic cells on the coated materials.
- The data can be used for further evaluation of parameters and functions of DLC coating. Properties of DLC change depending on the film thickness, optical properties, α-C:H conditions, density, sp^2 and hydrogen contents, and owing to the mechanotransduction or mechanosensor mechanisms of the cell system [6,7], it is presumed that the cell adhesion differs under the influence of such factors [3,8,9]. The current method as well as obtained data can be used for the evaluation of further coating development.

1. Data Description

Data were collected by experiments in which DLC was coated on the silicon plate surface, then mouse osteoblastic MC3T3-E1 and fibroblastic NIH3T3 cells were cultured with the coated plates. Colorimetric cell proliferation assays and morphological observation using a field emission scanning electron microscope (FE-SEM) were used to examine the proliferation and cell adhesion on the coated plates. The original data and conditions of each data are provided in the Mendeley Data repository (Manome, Yoshinobu; Tachibana, Toshiaki; Hiratsuka, Masanori; Sato, Keisuke; Ohgoe, Yasuharu; Hirakuri, Kenji (2021), "Attachment of Osteoblastic MC3T3-E1 Cells on Diamond-Like Carbon-Coated Silicon Plates", Mendeley Data, https://data.mendeley.com/datasets/yt52fw55wx.

2. Experimental Design, Materials and Methods

2.1. DLC coating on silicon plate surfaces

A parallel plate with a 13.56-MHz radio-frequency plasma enhanced chemical vapor deposition (RF-PECVD; PED-401, ANELVA Ltd., Japan) method was used to deposit the DLC film onto cut pieces (1 cm $\times$ 1 cm) of a silicon sheet (5*N*, Φ = 101.6 $\times$ 5-mm thickness, Kojundo Chemical Laboratory Co. Ltd., Saitama, Japan) placed on the cathode. This RF-PECVD method is one of the most common DLC film coating methods used in industrial settings [10]. The film deposition conditions were as follows: CH_4 gas with a flow rate of 37 sccm, pressure of 50 Pa, sputtering voltage of 200 V, and time of 2.2 min, and the resulting film thickness was 150 nm.

2.2. Cell cultivation

Mouse osteoblast MC3T3-E1 cells [5] obtained from the C57BL/6 mouse calvaria and mouse fibroblast NIH3T3 cells were obtained from American Type Culture Collection (ATCC, VA, USA).

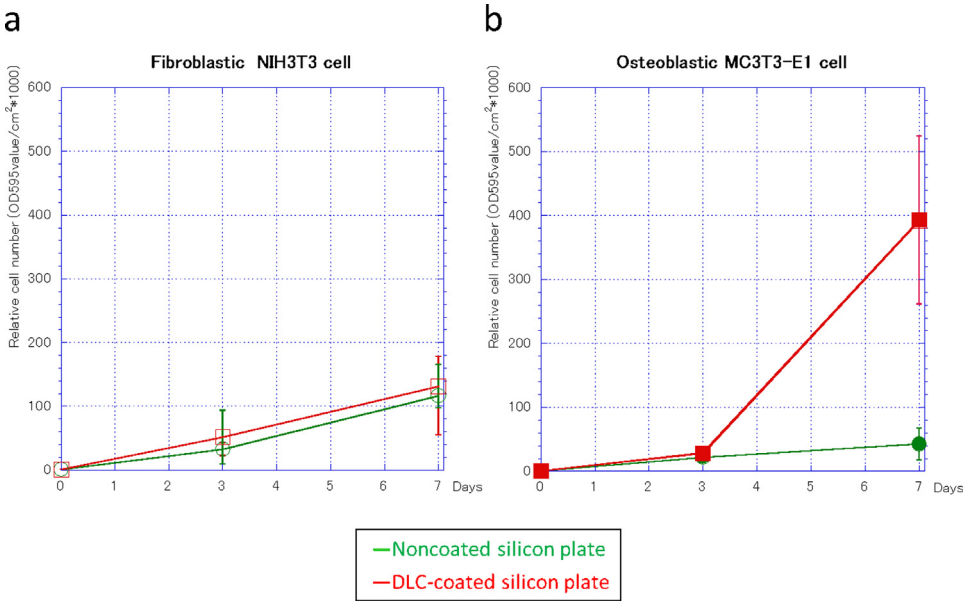

Fig. 1. Comparison of the proliferation of fibroblastic NIH3T3 cells (a) and osteoblastic MC3T3-E1 cells (b) on noncoated or DLC-coated silicon plates. After attachment of the cells on each plate, the proliferation of the cells was assessed on days 3 and 7 post-inoculation and compared. Proliferation was determined by colorimetric assays and each displayed data point is the mean value of three experiment, bars S.D. The raw data of this figure can be found in Mendeley Data (Fig. 1_data.xlsx and Fig. 1_additional_data.xlsx).

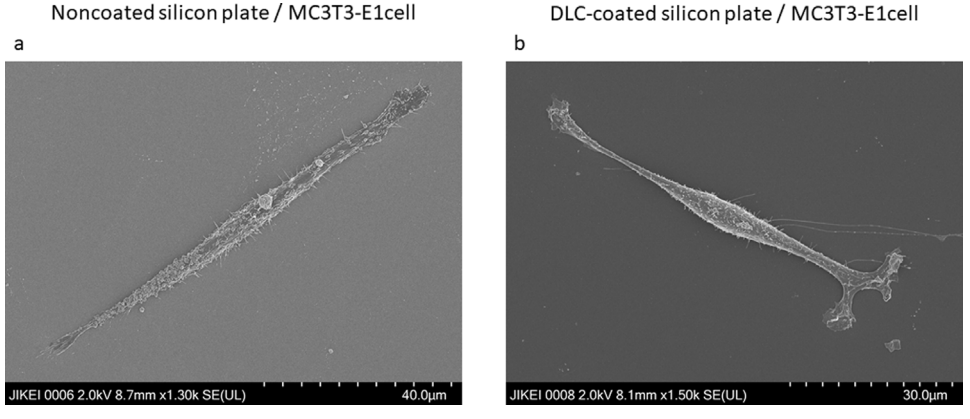

Fig. 2. FE-SEM images of MC3T3-E1 cells on noncoated (a) or DLC-coated silicon plates (b). The MC3T3-E1 cells adhered well on both the plates, but in a different manner. Filopodia and lamellipodia are clearly seen. Lamellipodia and filopodia of cells on noncoated silicon plate were not developed. In contrast, lamellipodia in cell on DLC-coated silicon plate were wider and length of filopodia reached more than 300 μm. The difference in background intensity is due to the different surface materials.

The cells were cultured in minimum essential medium (MEM)-α medium supplemented with 10% v/v fetal bovine serum and penicillin/streptomycin (100 IU/mL and 100 μg/mL; Sigma-Aldrich, MO, USA), and maintained until the day of the experiment.

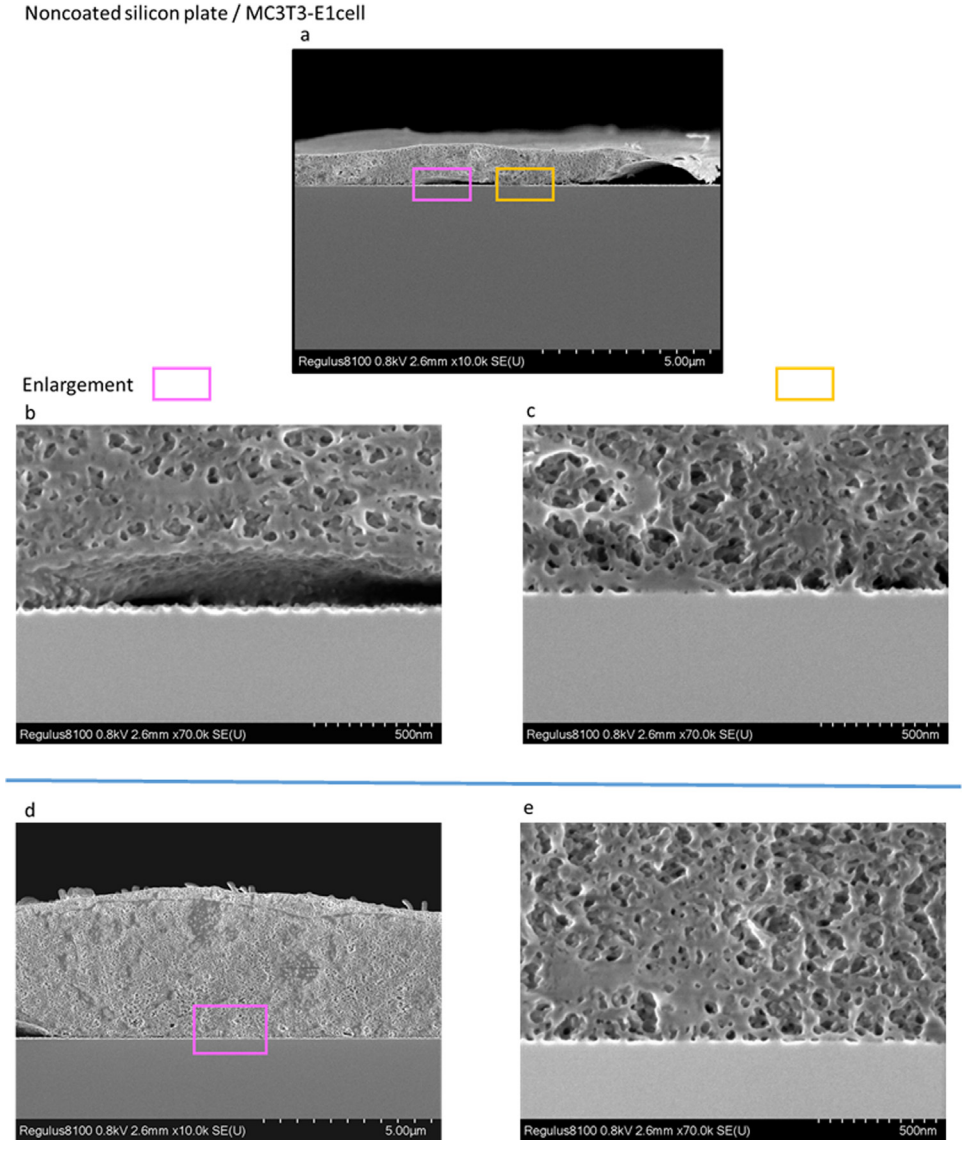

Fig. 3. Flat cross section of MC3T3-E1 cell on a noncoated silicon plate (a). To demonstrate the adherence of the cell onto the noncoated silicon plate, ion milling was conducted on the sample of the Fig. 2 (a) and flat cross section of the cell and plate was visualized with low and high magnifications. With the exception of unattached areas, the cell adhered well to noncoated silicon plate over a large surface area. Nevertheless, adhesion of cell body to the plate was not uniform due to the outline of the silicon plate surface. Observation under high magnification clarified the attachment of the cell. The pictures (b) and (c) are enlargements of the regions indicated in the picture (a). The picture (e) is an enlargement of the region indicated in the picture (d).

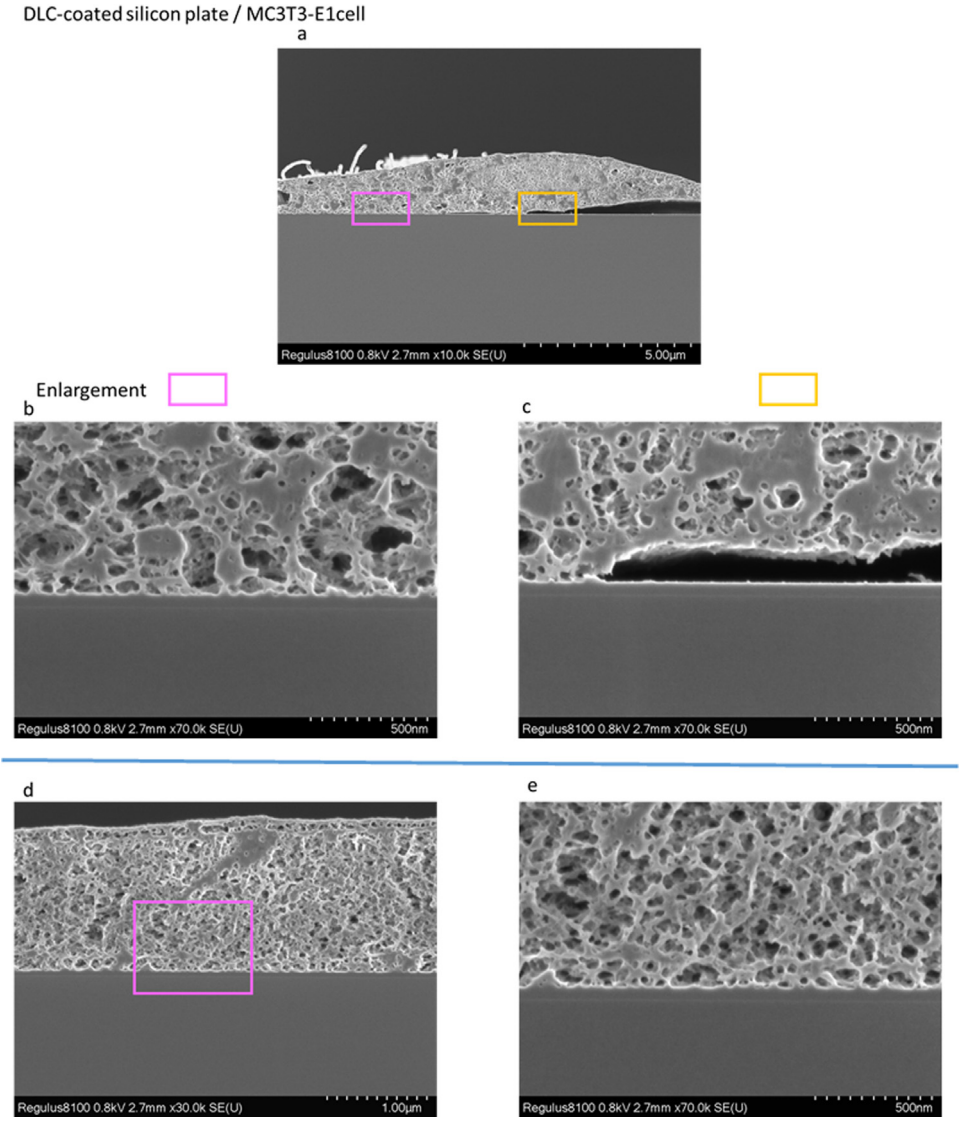

Fig. 4. Flat cross section of MC3T3-E1 cell on a DLC-coated silicon plate. The figure is a comparable counterpart of the Fig. 3. Similar to the Fig. 3, ion milling was conducted on the sample of the Fig. 2 (b) and flat cross section of the cell and plate was also visualized. Microvilli of approximately 5 to 10 μm were observed on the upper surface of the cell. The DLC coating was visualized in the flat cross section of the plate. The boundaries of DLC film to silicon plate and to the cell body were both flat and smooth. Compared to Fig. 3, adhesion of the cell to DLC coated silicon plate was tight, flat and uniform. The pictures (b) and (c) are enlargements of the regions indicated in the picture (a). The picture (e) is an enlargement of the region indicated in the picture (d).

2.3. Cell proliferation assay

Both cell lines (1.0×10^4 cells) were seeded on noncoated or DLC-coated silicon plates and cultured in Costar® 6-well cell culture plates (Corning, NY, USA) with the culture medium (3 mL).

After 3 and 7 days of being cultured, the cell proliferation was assayed by using a modified colorimetric assay described previously [11]. In brief, the cells were fixed on each plate by adding 25% glutaraldehyde (400 µL, Sigma-Aldrich) for 15 min. After washing three times with running water, the cells were stained with 0.05% methylene blue (1 mL) for 15 min. The dye was eluted with 0.33 N HCI for 15 min with agitation. The absorbance was measured using a microplate reader (680XR, Bio-Rad Japan Laboratory, Tokyo) at 595 nm. Values were determined to be within a linear range.

2.4. Morphological observation of the cells using FE-SEM

Cells on the noncoated or DLC-coated silicon plates were fixed with 1.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3, 400 mOsm/l.) for 1 h. After washing with 0.1 M phosphate buffer, the specimens were dehydrated with ethanol and a critical point dryer (HCP-2, Hitachi High-Tech Co., Tokyo, Japan). The plates were then coated with osmium (Os coater, HPC-1SW, Vacuum Device Inc. Ibaragi, Japan) and examined using FE-SEM(Regulus8100, Hitachi High-Tech Corp.). To obtain the flat cross section of the cells, ion milling (IM4000Plus, Hitachi High-Tech Corp.) was conducted on the same specimens as above. Milling conditions were as follows: accelerating voltage 4.0 kV, discharge voltage 1.5 kV, milling mode C4 $\pm$ 30°, milling time 1.5 h (noncoated plates) or 2.5 h (DLC-coated plates). Images were analyzed by WinROOF 2018 Image Analyser/Measurement software (Mitani Corporation, Tokyo, Japan).

Ethics Statement

The sample used in this study were silicon plates, DLC coating material and cells, therefore no ethical approvals were required for the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

CRediT Author Statement

Yoshinobu Manome: Conceptualization, Investigation, Writing – review & editing; **Toshiaki Tachibana:** Investigation, Methodology; **Masanori Hiratsuka:** Methodology; **Keisuke Sato:** Conceptualization; **Yasuharu Ohgoe:** Methodology, Resources; **Kenji Hirakuri:** Project administration, Conceptualization, Resources.

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