In vitro study of biocompatibility of several nanoporous micro silica particles used for controlled drug release

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In this study, we investigated in cytocompatibility and controlled drug-release property of several nano-porous micro silica (NPMS) particles. Though human alveolar epithelial cells (A549) and mouse osteoblastic cells (MC3T3-E1) were cultivated under NPMS exposure, they indicated excellent cell viability even the concentration and cultivation period increased. In contrast, cells exposed to CuO nanoparticles drastically decreased the viability depending on the concentration. In addition, NPMS contained dental materials specimen indicated sustained model drug release property more than 2 weeks. These results suggested that NPMS had excellent cytocompatibility and drug-release property. It can allow to apply for various bio/medical materials.

Key words: nano-porous micro silica particles, controlled drug-release, dental materials

1. INTRODUCTION

Because of their unique properties, nanomaterials have received much attention in a wide variety of fields [1-3]. For example, their surface area increases with decreasing size, their chemical reactivity increases. Particularly, nanostructured materials have very large surface area and their cavities can act as binding sites for some molecules or ions. Nano-porous micro silica particles (NPMS) were known to have sustained molecules release property because of their large surface area and nanostructured cavities. In these viewpoints, many researchers have investigated the synthesis of nanostructured materials, controlling the size and shape of the cavities, and their applications in several fields [4]. Someone have been reported that these nanomaterials can use as drug-release carriers. In our previous study, we investigated nanoporous silica particles (NPS) for the release of an antibacterial agent and calcium ion [5]. We also investigated the effect of electric charges on the NPS's surface on the capture and release behavior [6]. For fabrication these materials in biomedical fields, assessment of their biocompatibility is one of the most important viewpoint. Therefore, we investigated in their cytocompatibility in this study. This property can allow to fabricate widely fields. In this study, we investigated on cytocompatibility and drug-release property of NPMS.

2. Materials and Methods

2.1 Materials

Several types of NPSM and rhodamine B (RhB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dental glass ionomer cement GIC (Fuji I) was purchased from GC (Tokyo, Japan). The particles were characterized using S-4800 scanning electron microscopy (SEM: HITACHI, Tokyo, Japan) and JEM-1400 transmission electron microscopy (TEM: JEOL, Tokyo, Japan). TiO_2 and CuO microparticles were also purchased from Sigma-Aldrich.

2.2 Cytocompatibility

Human alveolar epithelial cells (A549) and mouse osteoblastic cells (MC3T3-E1) were

cultured in Dulbecco's MEM (Gibco, Carlsbad, CA, USA). These cells were seeded into 24-well plates at a density of approximately 1×10^4 cells/mL and incubated at 37°C in a humidified 5% CO_2 atmosphere for 48 h. Subsequently, the culture medium was aspirated and a fresh portion of the medium co ntaining nanoparticles was added. After 24h of incubation, cell viability was determined using the CellTiter-Glo assay kit (Promega, Madison, WI, USA), which measures intracellular adenosine triphosphate levels. The CellTiter-Glo reagent was added to the culture medium, and cell lysis was induced by shaking for 2 min. The luminescence signal was quantified using a microplate reader (Spectra Max Paradigm, Molecular Devices, Tokyo, Japan). The morphology of cells were investigated using a scanning electron microscope (SEM: S-4800, HITACHI, Tokyo, Japan)

2.3 Controlled drug-release property

The NPMS were mixed into GIC at 10 wt% concentration. Then specimens (GIC-NPS, ϕ10 x 1 mm) were prepared by the mixing. The GIC-NPS were immersed into 1 wt% RhB aqueous solutions at 37°C for 24h. Obtained GIC-NPS was immersed into distilled water at 37°C for 24h. The specimens removed to a fresh water every days for 28 days. Amount of released RhB into the supernatant were determined using UV-vis. spectrophotometer. After 28 days, the GIC-NPS was immersed into RhB solutions again for recharge of dyes. Then the release behaviour was estimated in the same manner.

3. Results and discussion

Figure 1 shows typical SEM images of several types of nano-poruos micro silica particles (NPMS).They have a spherical-shape and their diameters were approximately (a) 0.3-0.5 mm, (b) 0.5-0.8 mm, (c) 1 mm, and (d) 2 mm, respectively. These particles have an irregular structure in nano-size. Figure 2 shows the nano-structure of NPMS. Because of these structure, the surface area of the particle drastically increases.

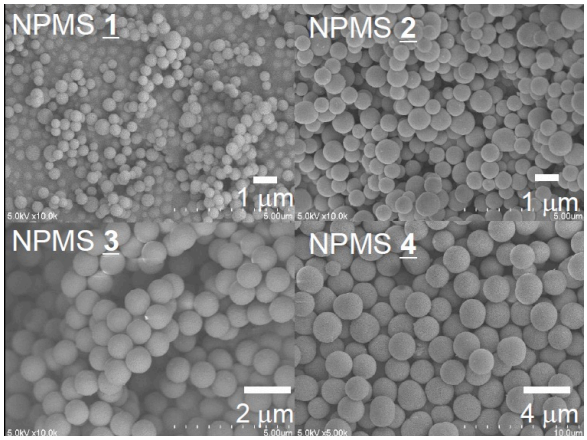

Figure 1 Typical SEM images of several NPMS (**1**), (**2**), (**3**), and (**4**)

To estimate the cytocompatibility of NPMS, A549 and MC3T3-E1 cells were cultivated under various concentrations of NPMS. As shown in Figure 3(a), even the concentration increased, the cell viability of A549 was remained until the concentration reached at 30 ppm. This behavior is almost same as that under TiO_2 nanoparticle exposure. In contrast, cell viability drastically decreased under CuO nanoparticles exposure depending on their concentration. In the case of MC3T3-E1 cells, they also almost same tendency under NPMS exposure. The viability was also estimated in viewpoint of time-dependence. As shown in Figure 3(b), A549 under NPMS exposure indicated high viability even after 3 days. These results indicated that NPMS has excellent cytocompatibility.

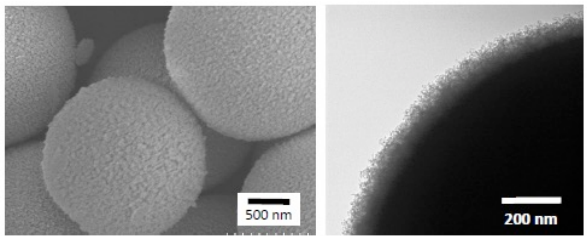

Figure 2. SEM (left) and TEM (right) image of NPMS(**3**).

For application of NPMS in bio/medical fields, their drug-release property were also investigated. NPMS were contained into GIC, which was a typical dental material, and model drug were charged to the specimens (NPMS-GIC). The obtained specimens were immersed in

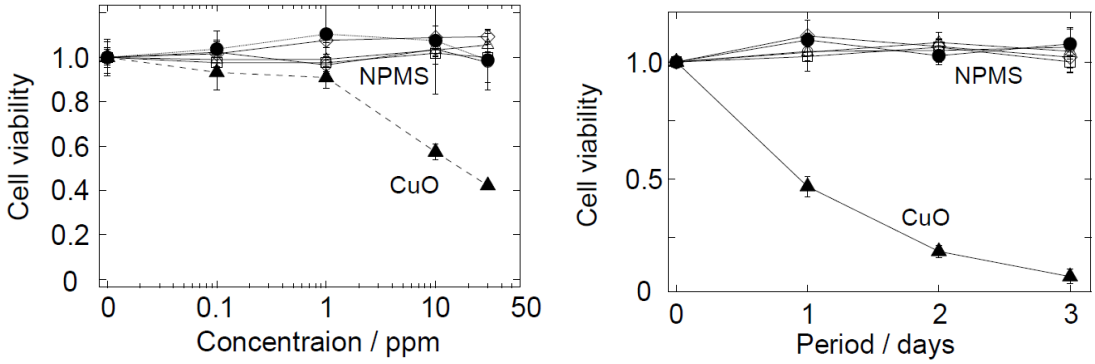

Figure 3. (a) Cell viability of A549 under NPMSs exposure with several concentration, (b) : time-dependent effect for cell viability under NPMS exposure (10 ppm); ◇: NMPS **1**, ○: NMPS **2**, □: NMPS **3**, △: NPMS **4**, ●: TiO_2, ▲: CuO.

distilled water. As show in Figure 4, RdB, used as a model drug in this study, was gradually released from NPMS-GIC. Even after 2 weeks immersion, release of RdB was observed. In contrast, GIC without NPMS (GIC only) can release only small amount of RhB and the release was observed a couple of days. These results suggested that NPMS can capture the model drug and release them gradually. RhB is a positive charged-molecule and silica surface is known to have negative charge [7]. Thus, the release property was yield to their electronic interaction based on large surface area of NPMS. In fact, NPS-GIC indicated low sustained-release property when anionic dye, such as flurosceine, was charged instead of RhB. The property in this case was similar to that of GIC only [6]. In addition, the property for anionic dye increased when NPS surface was modified using surfactant agents contained amine groups. Thus, NPMS can allow to apply for sustained cation release materials even a cationic drug molecule and the release property can be tuned using surface modification.

In this study, we investigated on biocompatibility and controlled drug-release behavior of nanoporous silica particles. Several sized micro silica particles shows excellent cytocompatibility and they can release cationic molecules for 2 weeks or more. The capture/release ability depends their surface electric property. These results suggest that NPS can be useful for controlled drug-release system.

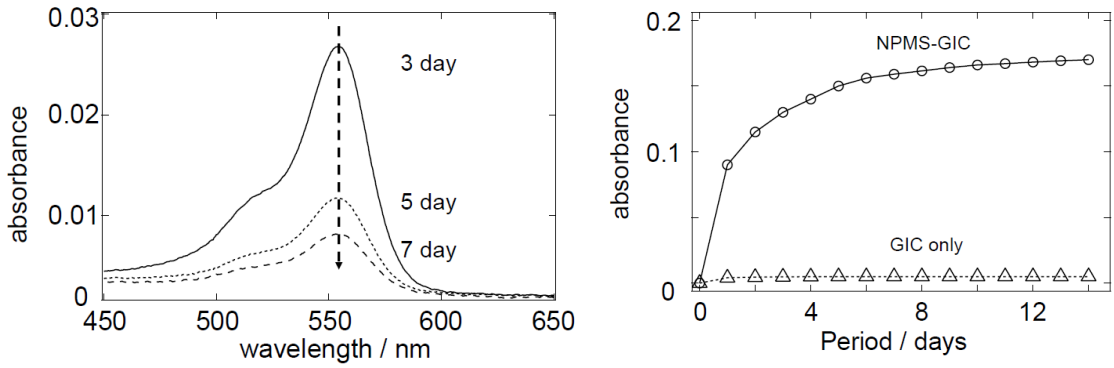

Figure 4 (a) Absorption spectra of released RhB in supernatant. (b) Time-dependent of RhB release behavior from NPMS-GIC (○), and GIC only (△).

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