Imaging of antibacterial activity of polymeric particles for drug delivery systems using scanning transmission electron microscopy

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[Introduction] Treatment methods using drug delivery systems (DDS) have been actively developed in recent years. We reported a preparation technique for submicrometer-sized, biodegradable poly(DL-lactide-co-glycolide) (PLGA) particles, which demonstrated controlled release, high stability, and enhanced absorption of drugs, making them promising DDS candidates [1]. Recently, biofilm infection such as periodontal disease, nosocomial infectious disease, and osteomyelitis became severe problems for human health. Once a biofilm is formed, it is difficult to remove it using antibacterial drugs because it is irreversibly associated with the surface and enclosed in a matrix of primarily polysaccharide material. To optimize the preparation technique of DDS particles for biofilm infections, we need to visualize an understand interaction between polymeric NPs and biofilm. Thereby, we developed sample pretreatment technique using an ionic liquid (IL) and scanning transmission electron microscopy-cathodoluminescence (STEM-CL).
[Materials and methods] *S. epidermidis* was used as a model biofilm forming bacterial strain. The bacterial cells were grown in a conical tube at 37°C for 24 h under 0.5% CO_2 in tryptone soy broth supplemented with 0.25% glucose. The IL used for STEM observation was 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]). STEM observation was carried out by JEM-2100M or JEM-2100F with a cooling holder. A STEM equipped with a Gatan Vulcan CL system Model 465, a suite of a single-tilt liquid nitrogen-cooling holder with a light detection system and a spectrometer, was used for the CL analyses as shown in Fig. 1. The ultrathin section method was applied for sample preparation of TEM observation (JEM-1400Plus). Polymeric particles were prepared using base polymer such as PLGA and polyvinyl caprolactam - polyvinyl acetate - polyethylene glycol graft copolymer (Soluplus, Sol) by the emulsion solvent diffusion method. Clarithromycin (CAM) and chitosan (CS) or metal nanoparticles were used for antibacterial drug and surface modifier for particles. Antibacterial assays were performed using the LIVE/DEAD BacLight bacterial viability kit.
[Results and discussion] We have demonstrated the feasibility of STEM-CL imaging of DDS polymeric particles-treated biofilms (Fig. 2). The sample pretreatment technique using an IL + STEM-CL imaging and conventional imaging using the ultrathin section method revealed different antibacterial activity of various kinds of polymeric particles at a nanoscale level (Fig. 3). It was found that a combination of the base polymer (PLGA or Sol), antibacterial drug (CAM), and surface modifier (CS or metal nanoparticles) can make suitable DDS polymeric particles for treating the biofilm infections. Especially, CL imaging and CL analysis of polymeric particles in the biofilm was successfully performed by attaching the fluorescent materials. The results of an antibacterial assay, as well as the physicochemical properties of particles, showed that there was no significant difference between fluorescent materials attached polymeric particles and polymeric particles treatments with regard to modulation of a biofilm. These developed techniques could be effective to design of polymeric particles for treating biofilm infection diseases.

[Acknowledgement]
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[Reference]
[1] Takahashi *et al.*, RSC adv., 5 (2015) 71709 - 71717.

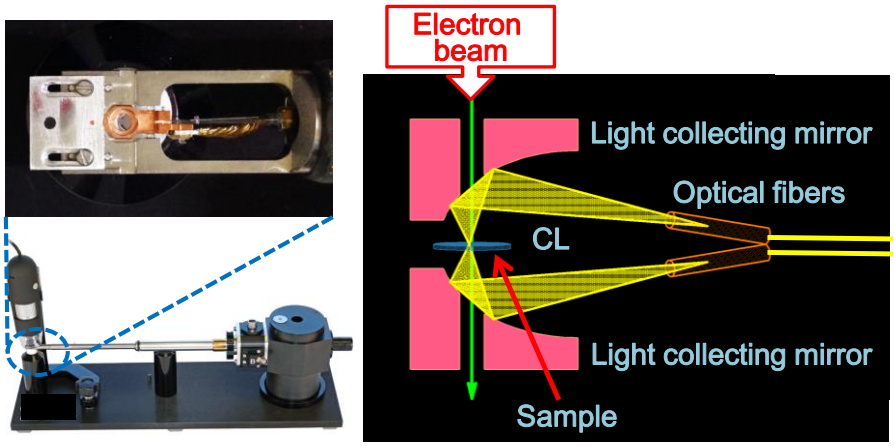

Fig. 1. Schematic image for the tip of the cryo holder and STEM-CL system.

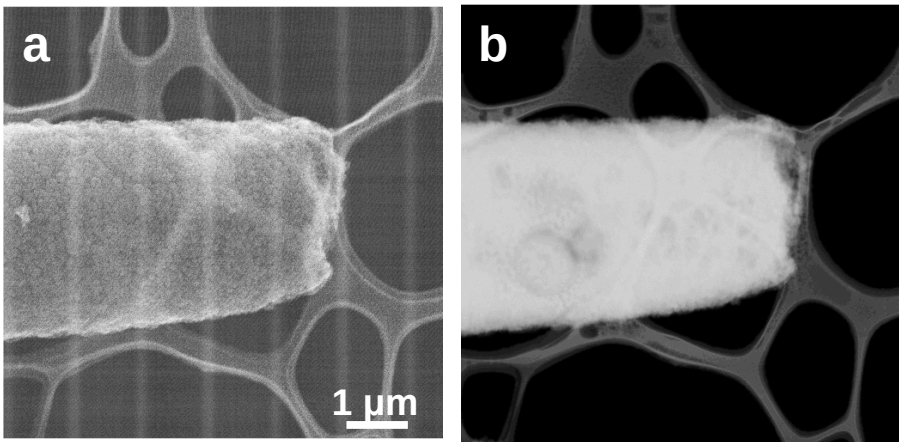

Fig. 2 SEI-COMPO image (a) and CL image (b) of fluorescent material attached chitosan modified PLGA polymeric particles.

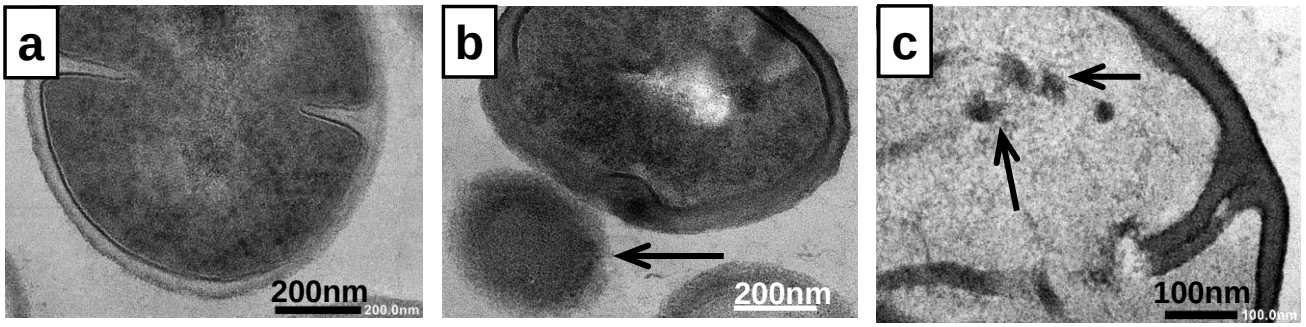

Fig. 3 TEM images of the biofilm (a), biofilm treated with drug incorporated + chitosan modified PLGA particles (b) and drug incorporated + chitosan modified Sol particles (c). The arrows in Figure indicate polymeric particles.