Characterization of Antibacterial Nanoparticles from the Scallop, *Ptilopecten yessoensis*

Mi Suk Jeong,1 Jeong Soon Park,2 Seong Hwan Song,1 and Se Bok Jang2,1

1Research Center for Advanced Science and Technology, Dongseo University, Busan 617-716, Korea
2Department of Molecular Biology, College of Natural Sciences, Pusan National University, Jangjeon-dong, Geumjeong-gu, Busan 609-735, Korea

Received April 17, 2007; Accepted June 15, 2007; Online Publication, September 7, 2007

To develop a new drug delivery system, antibacterial 50–900 nm nanoparticles of shell and internal organs from scallops collected off Huksan-Island, Korea, were prepared by dry grind technology respectively. The diameters, identities, and conformations of the scallop shell and internal organ particles were determined with a particle-size analyzer and by X-ray powder diffraction (XRD), scanning electron microscopy (SEM), and Raman spectroscopy. The antibacterial properties of the nanoparticles from scallop shell were investigated in the absence and the presence of scallop-shell extract. Bacterial growth was reduced with the supernatant of the nanoparticle scallop-shell extract. Also, the nanoparticles from scallop shell were much more effective as a skin softener than was powder. These facts provide us with guidelines for the study of the size-dependent properties of functional materials as well as for further applications to drug delivery systems (DDSs) and cosmetic raw materials.

**Key words:** antibacterial nanoparticles; scallop; drug delivery system

Natural scallops entirely free of pollution are among the major marine products of Huksan Island, Korea. We have been engaged in research to utilize scallop shells efficiently as a useful natural resource. Scallop shells have been used as a source of CaCO$_3$ and organic compounds, and as material for desulfurization, and their further utilization is currently desirable. Liu et al. have studied the *in vitro* activities of the components of scallop shells, and have an ability to protect the skin. A scallop-shell water extract showed the growth-promoting activity of skin fibroblast cells and skin keratinocyte cells, and inhibited production of the superoxide anion generated by the reaction of xanthine and xanthine oxidase. In addition, the extract heightened the rate of recovery from UV-induced injury *in vivo*. To increase the efficaciousness of scallop-shell powders in skin disease and for beauty, we prepared antibacterial nanoparticles from the shells. These properties indicate new applications for scallop shells.

Recently, increasing attention has been focused on formulating therapeutic agents in biocompatible nanocomposites such as liposomes, nanocapsules, micellar systems, and conjugates. Since these systems are often polymeric and submicron in size, they can in general be used to provide targeted delivery (cellular/tissue) of drugs, to improve bioavailability, to sustain drug effects in target tissues, to solubilize drugs for intravascular delivery, and to improve the stability of therapeutic agents from enzymatic degradation.

Nanoparticle characterization of CaCO$_3$ from scallop shells and internal organs was done with a particle-size analyzer, and by scanning electron microscopy, X-ray powder diffraction, and Raman spectroscopy. The antibacterial properties of nanoparticles from scallop shells were investigated.

**Materials and Methods**

**Size measurement of scallop nanoparticles.** Nanosized particles from scallop shells and internal organs were prepared with the Nano Grinding Mill System (Nanotech World, Pohang, Korea). Particle mean diameter was determined with the Particle Size Analysis System (UPA-150, Microtrac, Montgomeryville, PA). The analysis was performed at a scattering angle of 90° and at a temperature of 25°C using samples appropriately diluted with ultrapurified water.

**Particle morphology.** The morphology of the nanoparticles, which were prepared on aluminum stabs and coated with gold, was determined using SEM (Hitachi S-3500N). The magnification produced by SEM is the ratio between the dimensions of the final image display and the field scanned on the specimen. Usually the SEM magnification range is between 10 and 200,000X.

---

1 To whom correspondence should be addressed. Tel: +82-51-510-2523; Fax: +82-51-581-2546; E-mail: sbjang@pusan.ac.kr
Powder X-ray diffraction (XRD). X-ray diffractometry (X’pert PRO MRD, Philips, Tokyo), was used in diffraction studies. The XRD studies were conducted by exposing samples to Cu Kα radiation (40 kV, 20 mA) and scanning them from 2θ to 70θ at 2θ at increments of 0.02° and 5 s duration.

Raman spectroscopy. Raman spectra of the scallop shells were obtained using a Confocal Raman microspectrometer (Nanofinder 30; Tokyo Instruments, Tokyo). This spectrometer was coupled with an optical microscope (Nikon TE2000-U) and an air cooled charge-coupled device (CCD) detector. Raman scattering was excited by the 488 nm line from a Sapphire laser (by the 632.8 nm line from a He–Ne laser). The laser beam was focused on a sample using a 40x microscope objective. The laser power at the sample was about 3 mW. The Raman spectra were recorded in the 150–3,500 cm⁻¹ wave range, with an exposure time of 60 s.

Through a pinhole 35 μm in diameter, the spectral resolution was 1.1 cm⁻¹.

Antibacterial tests. The antibacterial properties of the nanoparticles from scallop shells were investigated. Two LB agar plates scratched with finger were incubated at 37°C for 1 d in the absence and the presence of scallop-shell extracts. The type of inoculated bacteria was identified by the gram stain method, the catalase test, and with an API biochemical kit according to the instructions of the manufacturer (bioMérieux, Marcy-l’Etoile, France).

Results and Discussion

Nano-sized particles from scallop shells and internal organs were prepared by dry grind technology. The size and size distribution of the nanoparticles were determined using a particle-size analyzer. The particles were
in the range of 75–1,606 nm in diameter (Fig. 1A and B). The diameter and width were 1,606 nm and 471 nm for the shells and 75 nm and 368 nm for the internal organs.

SEM observations showed that in one of the cases the nano-sized scallop particles exhibited irregular sphericity in the shells and a stacking layer (Fig. 2A and B). Surfactants were used to disperse the scallop shell and internal organ powders. SEM images of the resulting samples revealed a distribution of extended nanoparticles. The average length and root-mean-square end-to-end distance as measured from the SEM images were 75 and 1,606 nm respectively. The samples without surfactant showed a strong tendency to form aggregates. These aggregates were visualized by SEM. The particle-size distribution obtained with the laser particle size analyzer was confirmed from SEM images. The nanoparticles were rigid and spherical, as determined by SEM and particle size analysis.

The nanosize scallop-shell clusters were dispersed in pure water. The clusters thus re-formed were of the pure water phase, and their sizes correlated with the absorption force. In the case of a high absorption force, the nano-particles percolated through the skin cells. These particles were mostly 50–900 nm in size. Larger-sized clusters were dispersed slowly in pure water. These scallop-shell particles, according to their size, exhibited an absorbance behavior different from that of bulk scallop shell. The microenvironment for surface sites is different from that of bulk. The particle size analysis
results showed that whereas smaller-sized particles were confined mostly inside the pore system, the larger-sized particles occupied the external surface of the host matrix.

In the present study, scallop-shell powder was exposed to heat treatment of between 1,200 and 1,300°C for 3–4 h to remove the poison. The main component of scallop-shell powder is calcium carbonate (CaCO$_3$). Through heat treatment, CaCO$_3$ in the shell is converted to CaO, which exhibits antibacterial activity. The bactericidal action of the powder has recently been investigated. Shell powder heated to 700°C or higher exhibited bactericidal action against Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, and Bacillus subtilis. An increase in exposure temperature enhanced that bactericidal action, which springs from the conversion by heat treatment of calcium carbonate, the main component of scallop-shell powder, to calcium oxide. Such results can be applied in experimentation, and helped in our case. The scallop-shell nano-particles were characterized by XRD analysis (Fig. 3). The results revealed the presence of CaCO$_3$ in the scallop shells. It consisted 98% of calcium carbonate and 2% of organic compounds. The XRD pattern of scallop-shell nanoparticles showed that the intensity and the distance between crystal planes of diffraction peaks of the nanoparticles were in good agreement with the standard spectrum of the synthetic calcite of calcium carbonate (ICDD #05-0586).

Raman spectra (B–F) were recorded in the 150–3,500 cm$^{-1}$ wave range at an exposure time of 60 s (Fig. 4). There were three discernible Raman bands between 0 and 3,200 cm$^{-1}$ in natural scallop shells. The Raman spectrum of the CaCO$_3$ particles as calcite exhibited the strongest CO stretch band at 1,088 cm$^{-1}$. The mode appeared as a weak band in the spectrum at 1,098 cm$^{-1}$, and was split into two bands near 1,088 cm$^{-1}$. As expected, the Raman band was observed at 300 cm$^{-1}$, appearing as a weak, sharp band. The broad band at 1,575 cm$^{-1}$ was observed to be much more shifted than the data on the spectra of carbonate in the literature. A strong doublet at 1,088 cm$^{-1}$ was unshifted, but two bands were shifted up in the spectrum to 300 and 1,575 cm$^{-1}$. Calcium carbonate Raman peaks at
280, 712, 1,088, and 1,436 cm\(^{-1}\) are commonly observed.\(^{16}\)

The main component of scallop-shell powder, in fact, is calcium carbonate. Through heat treatment, CaCO\(_3\) in the shell is converted to CaO, which exhibits antibacterial activity. The disinfecting effect of heated scallop-shell nanopowder on athlete’s foot and the hand was investigated. In the absence and the presence of scallop-shell extracts, two LB agar plates scratched with finger were incubated at 37°C for 1 d. Bacterial growth was reduced by the supernatant of the nanoparticle scallop-shell extract (Fig. 5A and B). The concentrations of nanoparticles scallop-shell extract on the LB plates were 40 and 60 mg/ml. The type of inoculated bacteria was identified as \textit{Staphylococcus epidermidis} by the gram stain method, and the catalase test, and with an API biochemical kit (bioMerieux).\(^{12}\) Scallop-shell powder treatment was found to reduce the aerobic bacteria count in athlete’s foot and on the hand, with increasing effectiveness at higher nanopowder concentrations. An instance of athlete’s fungus was eliminated within 20–30 min by treatment with as little as 100–900 nm of powder (data not shown). We postulate that a fraction of bacterial cells in the initial population becomes tolerant to the shell powder. A proposed model accurately predicted the reduced scallop bacterial counts by scallop-shell powder treatment. In that study, the antifungal activity of scallop-shell powder, heated to 1,000°C for 1 h against \textit{Trichophyton}, was kinetically investigated, and the possibility of applying the powder to the treatment of dermatophytosis was examined.\(^{15}\) The death rate of athlete’s foot fungus in heated shell-powder slurry increased with powder concentration, following first-order reaction kinetics. The trial, using heated shell powder treatment on the feet, showed the possibility of the powder’s application to the treatment of dermatophytosis.

The efficacy of the delivery system was evaluated for the mean size, size range, surface roughness and porosity, sphericity, and clumping of microspheres by optical microscopy and particle size analysis. It can be concluded that, with regard to the size and morphological characteristics of the prepared microspheres and their ability to preserve the antigenicity of the encapsu-
lated nanoparticles, they can be used as a nano-particle delivery system.

Acknowledgment

The authors acknowledge Dae-il Kang (Nanotech World) and Kyu Suk Choi (KSBI) for assistance. This study was supported by the Korea Research Foundation Grant Program, funded by the Korean Government (MOEHRD), KRF-2005-041-E00510 (to S.B.J.) and KRF-2005-075-C00019 (to M.S.J.).

References


Fig. 5. Antibacterial Tests of Nanoparticles from Scallop Shell.

In the absence (left) and the presence (right) of scallop-shell extracts, two LB agar plates scratched with finger were incubated at 37°C for 1 d. The concentrations of nanoparticle scallop-shell extract on LB plates were 40 mg/ml (A) and 60 mg/ml (B). Bacterial growth was reduced by the supernatant of the nanoparticle scallop-shell extract.