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Preparation and characterization of poly(oligo(ethylene glycol) methacrylate)-*grafted*-layered double hydroxides by RAFT polymerization

Thi Kim Lien Nguyen[a], Thanh Luan Nguyen[b], Xuan Thang Cao[a], Chan Park[c], and Kwon Taek Lim[a]

[a]Department of Display Engineering, Pukyong National University, Busan, Korea; [b]Department of Aquatic Life Medicine, College of Fisheries Science, Pukyong National University, Busan, Korea; [c]Department of Materials Engineering, Pukyong National University, Busan, Korea

ABSTRACT

The composite material based on poly(oligo(ethylene glycol) methacrylate)-grafted-layered double hydroxides/Gentamicin (LDHs-g-POEGMA-GE) was prepared by the reversible addition fragmentation chain transfer polymerization, and its antibacterial property was investigated. Initially, the surface of LDHs was modified by S′-(3-trimethoxysilyl) propyl trithiocarbonate as a chain transfer agent and then grafted with POEGMA. Subsequently, GE was covalently linked to the POEGMA chains by esterification reaction producing LDHs-g-POEGMA-GE. The composites were characterized by using FT-IR, GPC, XRD, TGA, and SEM analyses. The in-vitro assay with Staphylococcus aureus bacteria indicated the high antibacterial activity of the composites, which promises potential applications in the biomaterial field.

KEYWORDS

LDHs; Poly(oligo(ethylene glycol)methacrylate; Gentamicin; Antibacterial

Introduction

Layered double hydroxides (LDHs), an extraordinary class of layered materials with positively charged brucite-type inorganic layers and interlayers concept charge-balancing anions and solvation molecules, have attracted much attention recently [1]. In the geometric structure of this material, the metal cations fill the center of octahedral units to form infinite 2D sheets by edge-sharing and the vertexe of per units contains the hydroxide ion perpendicular to the plane of the layers. In addition, the hydrogen bonds inside the gallery of the LDHs between the hydroxyl groups and anions create their own chemical versatility and anion exchange capability, which allows fine tuning of their composition and properties in a wide range. The LDHs became one of the most favorable layered crystals for preparation of multifunctional polymer/layered crystal nanocomposites [2, 3], which have bseen used as a drug carrier, cellular delivery, and biomolecule protector systems by introducing the drugs or polymer chains into the narrow gallary space. However, these applications require a complicated process. The surface of the LDHs could be functionalized to carry macromolecules by controlled living polymerization in order to overcome this, which possibly controls molecular weight and narrow

CONTACT Kwon Taek Lim ktlim@pknu.ac.kr Department of Display Science & Engineering, Pukyong National University, Busan, 608-737, South Korea.
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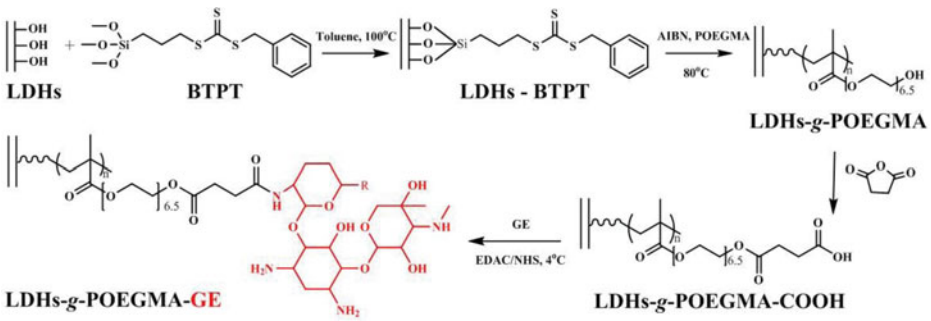

Figure 1. Schematic representation for the synthesis of LDHs-*g*-POEGMA-GE composites.

polydispersity [4–6]. Poly(oligo(ethylene glycol)methacrylate) (POEGMA), one of the most excellent candidates, is used to conjugate with the LDHs due to its nontoxicity, biocompatibility, and solubility in polar solvents. Especially, POEGMA has many oligos(ethylene glycol) side chains, which stabilize against aggregation and prevent protein and bacterial adhesion [7–9]. In addition, Gentamicin (GE), with a bactericidal mode of action through binding to the ribosome and various ribozymes, is used to treat many types of bacterial infections. The combination of these materials is promising an advanced composite for diversity applications.

In this study, the composite of poly(oligo(ethylene glycol) methacrylate)-*grafted*-layered double hydroxides/Gentamicin (LDHs-*g*-POEGMA-GE) was prepared by the reversible addition fragmentation chain transfer (RAFT) polymerization and esterification reaction (Fig. 1). The composites were characterized and illustrated antibiotic activity against gram-positive bacteria.

Experimental

Materials

OEGMA (M_n = 360) were purified by passing neutral aluminum oxide column. Gentamicin sulfate salt, calcium nitrate tetrahydrate (99%), aluminum nitrate nonahydrate (98%), (3-Mercaptopropyl)trimethoxy silane (95%), carbon disulfide (99%), benzyl bromide (99%), sodium hydroxide (97%), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDAC), succinic anhydride (SA, 99%), 4-(Dimethylamino)pyridine (DMAP), phosphate buffered saline (PBS, pH7.4), and N-Hydroxysuccinimide (NHS) were used as received. 2,2′-Azobis(2-methylpropionitrile) (AIBN, 98%) was recrystallized in methanol. Toluene, pyridine, dichloromethane (DCM), methanol, tetrahydrofuran (THF) were purified by distillation before use. All chemicals were purchased from Sigma-Aldrich (Korea). LDHs were prepared according to the method decrisbed in the previous literature [10]. S′-(3-trimethoxysilyl) propyl trithiocarbonate (BTPT) was prepared by using the previous procedure [11].

Anchoring of BTPT onto LDHs surface (LDHs-BTPT) and synthesis of LDHs-g-POEGMA by RAFT polymerization

A mixture of 0.7 g of LDHs and 20 ml of dry toluene was stirred in a round bottom flask at 100°C. Then, 0.724 g (2 mmol) of BTPT in dry toluene (10 ml) was injected into the flask under nitrogen. The reaction was conducted for 24 h. The crude product was filtered off and washed with DCM for three times to remove all unreacted BTPT. LDHs-BTPT was dried

under vacuum overnight. LDHs-*g*-POEGMA was prepared as follows: 2.0 g of OEGMA, 0.3 g of LDHs-BTPT, 0.020 g of AIBN, and 4 ml of dry toluene were added in a round bottom flask. Then, the reaction mixture was purged under nitrogen and stirred at 80°C for 24 h. The mixture was precipitated in diethyl ether and washed three times with methanol. The product was dried under vacuum at 40°C overnight (yield, 60%). The POEGMA brushes were cleaved from the LDHs using aminolysis reaction for gel permeation chromatography analysis [12], ($M_{n,GPC} = 4500$ g/mol, PDI = 1.39).

Preparation of LDHs-g-POEGMA-GE composites

Firstly, the hydroxyl terminated POEGMA was converted to carboxyl groups using ring-opening reaction of SA. In a typical procedure, 0.30 g of LDHs-*g*-POEGMA, 0.30 g of SA (3 mmol), 0.122 g of DMAP (1 mmol), 0.079 g of pyridine (1 mmol), and 5 ml of THF were added into a round bottom flask. The mixture was stirred at 50°C for 24 h. The product was filtered off, washed with DCM (3×10 ml), and dried in a vacuum oven. The reaction conversion was calculated by back titration to determine the number of carboxylic acid groups. The preparation of LDHs-g-POEGMA-GE composites were similar to the previous report [13]. Then, 0.10 g of carboxylic bearing LDHs-*g*-POEGMA was reactivated using a mixture of 0.018 g of NHS and 0.18 g of EDAC in 10 ml of PBS for 1 h. GE (0.135 g) in 5 ml of PBS was added dropwise to the mixture. The esterification reaction was allowed to take place at 4°C for 24 h. The product was washed with PBS (10 ml) and water (3×10 ml). The final product was freeze-dried and stored under 4°C.

Antibacterial activity experiment

The bacteria *Staphylococcus aureus* KCCM 40881 (Korean Culture Center of Microorganisms) was used for the antibacterial assay. It was inoculated with a 10 ml of sterile Mueller-Hinton (MH) broth medium and incubated overnight at 28°C in shaking incubator. The bacterial inoculum (108 CFU/mL) was then uniformly spread using a sterile cotton swab on a sterile Petri dish MH agar. The agar wells were prepared by 6 mm diameter holes borer. The in-vitro test was conducted with four samples. The PBS solution was a controlled sample. The reference commercial antibiotic sample was composed by dissolving GE in 2 ml of PBS with the same amount of GE for the carbodiimide reaction. 100 mg of composites in 2 ml of PBS was tested with bacteria. 50μl of the sample was put into the wells and incubated at 28°C for 24 h before measuring the diameter of inhibition zones.

Characterization

Gel permeation chromatography (GPC) was performed using an HP 1100 apparatus, with THF as a solvent at 25°C. The columns were calibrated with commercial polystyrene standards. Fourier transform infrared (FTIR) spectra were measured on a JASCO FT/IR-4100 spectrometer with a DLATGS detector. The crystallographic state of the nanocomposites was studied by a Philips X'pert-MPD system diffractometer. Thermogravimetric analysis (TGA) was conducted with a Perkin-Elmer Pyris 1 analyzer (USA). The morphology analyzes of the hybrids were carried out by using scanning electron microscopy (SEM) images equipped with an energy dispersive X-ray (EDX) spectrometer (Hitachi JEOL-JSM-6700F system, Japan).

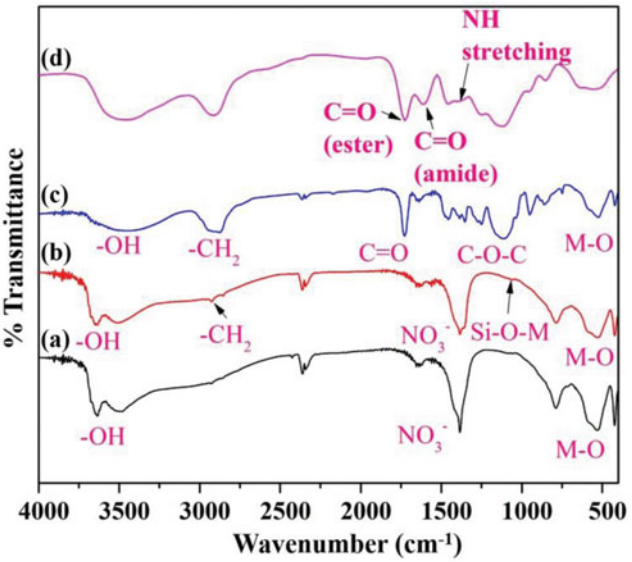

Figure 2. FT-IR spectra of (a) LDHs, (b) LDHs-BTPT, (c) LDHs-*g*-POEGMA, and (d) LDHs-*g*-POEGMA-GE composites.

Result and discussion

Preparation of LDHs-g-POEGMA

Figure 2 depicts the FT-IR spectra of composites. The spectrum of pure LDHs showed a broad band from 3400 to 3700 cm^{-1} due to the -OH stretching vibration (Fig. 2(a)) [14]. The strong peak at 1384 cm^{-1} was attributed to the interlayer NO_3^- while the broad peaks from 788 to 530 cm^{-1} were implied to M–O and O–M–O vibrations in the LDHs structure. After modification with the RAFT agent, the LDHs showed the characteristic peaks at 2924 and 1060 cm^{-1} owing to methylene and Si-O-Si vibrations (Fig. 2(b)). As expected, LDHs-*g*-POEGMA presented new absorption bands at 1729, 1251, and 1114 cm^{-1} indicating the stretching vibration of C = O and C-O-C of POEGMA moieties (Fig. 2(c)). The formation of the LDHs-*g*-POEGMA-GE was illustrated by the FT-IR spectrum in Fig. 2(d). It is clearly seen that the new bands at 1630 cm^{-1} (C = O amide bond vibration) and 1413 cm^{-1} (NH stretching) demonstrated the anchoring GE onto the LDHs-*g*-POEGMA.

Figure 3 shows the XRD patterns of LDHs and composites. It is obviously observed that the peaks belonging to LDHs-*g*-POEGMA (Fig. 3(b)) and the LDHs-*g*-POEGMA-GE (Fig. 3(c)) are perfectly indexed to tetragonal phase of the LDHs which present 2θ values of 11, 23, and 31 corresponding to the crystal planes of (003), (006), and (110), respectively (Fig. 3(a)). The XRD result revealed the grafting of the polymer did not alter the crystallinity of the LDHs.

TGA was performed to examine thermal degradation. As shown in Fig. 4, LDHs started to decompose at 110°C and lost 24% of weight due to the release of absorbed water and interlayer anions (Fig. 4(a)). LDHs-BTPT began to degrade at 80°C and the weight loss is 24% at 800°C since the encapsulating organic molecules decamped at high temperature (Fig. 4(b)). The as-prepared LDHs-*g*-POEGMA were significantly degraded between 280 and 430°C and lost 90% at 800°C (Fig. 4(c)). Therefore, the LDHs-*g*-POEGMA possess higher starting degradation temperature in comparison with LDHs and LDHs-BTPT due to the coverage of polymer

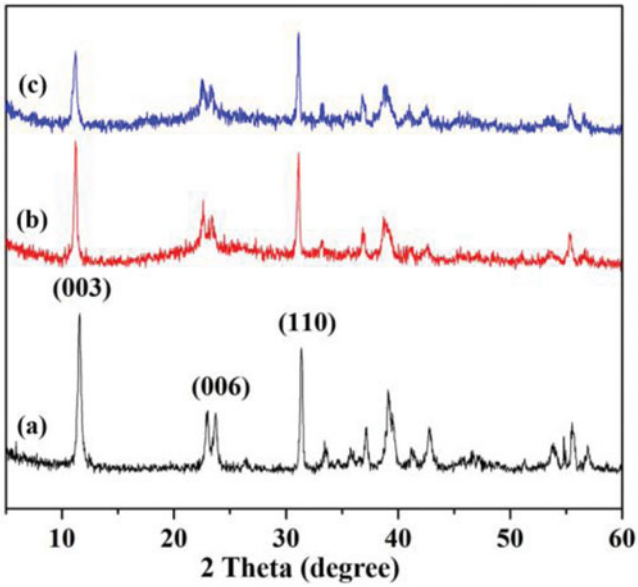

Figure 3. X-ray diffraction patterns of (a) LDHs, (b) LDHs-BTPT, and (c) LDHs-*g*-POEGMA.

layers which prevent the escape of absorbed water and interlayer anions. The TGA results demonstrated that the brush polymers were successfully decorated onto the LDHs.

The surface morphologies of pristine LDHs and LDHs-*g*-POEGMA could be visualized by using SEM characterization. The morphology of LDHs displayed the crystalline structure as shown in Fig. 5(a). After RAFT polymerization, LDHs-*g*-POEGMA image showed relatively irregular shape having a soft layer due to the POEGMA chains (Fig. 5(b)).

In-vitro results

The hydroxyl groups of LDHs-*g*-POEGMA was converted to carboxylic groups with 22% of conversion as indicated by back titration. Then, GE was attached to the composite by the

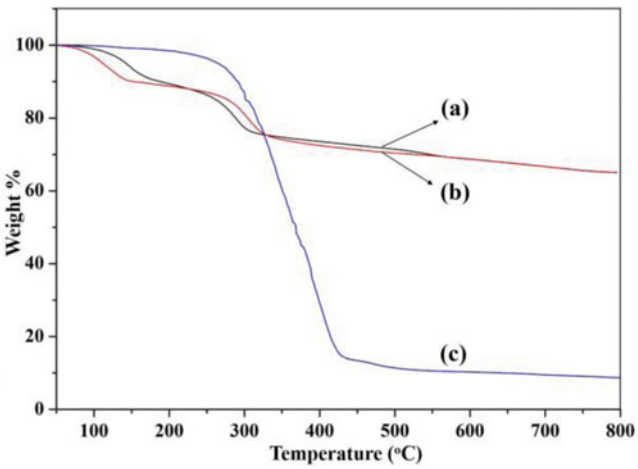

Figure 4. TGA spectra of (a) LDHs, (b) LDHs-BTPT, and (c) LDHs-*g*-POEGMA.

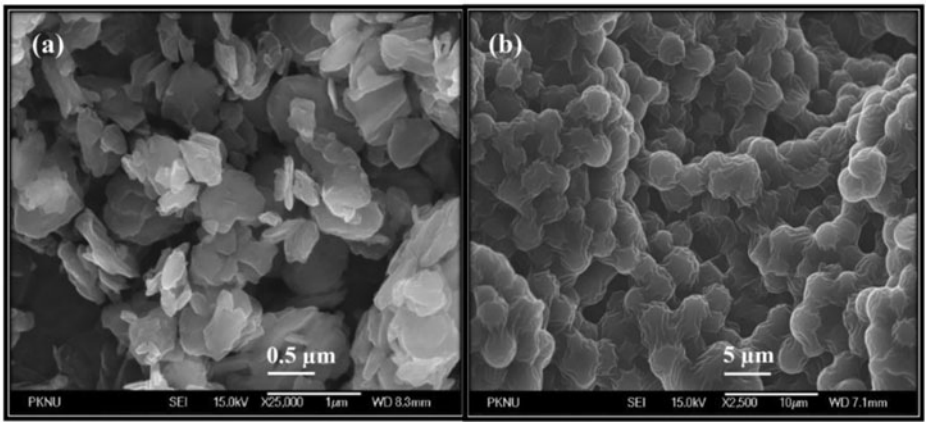

Figure 5. SEM pictures of (a) LDHs and (b) LDHs-*g*-POEGMA.

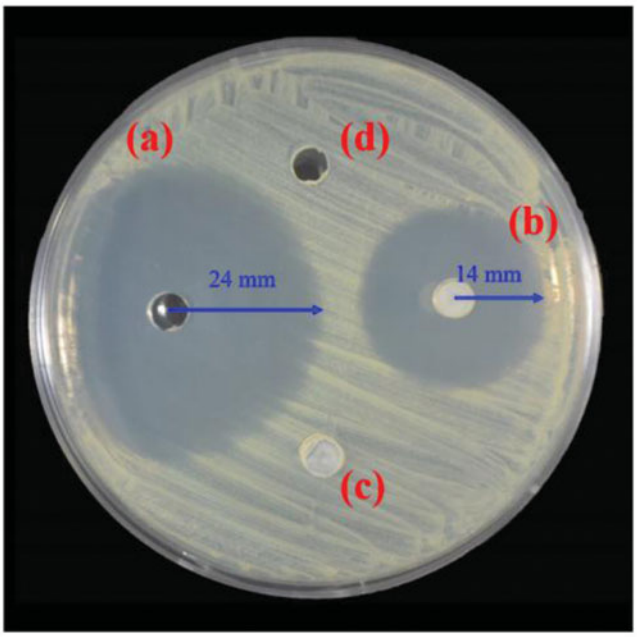

Figure 6. The *in-vitro* test with *Staphylococcus aureus* bacteria after incubating at 28°C, 24 h: (a) pristine GE, (b) LDHs-g-POEGMA-GE, (c) LDHs-g-POEGMA, and (d) blank sample.

carbodiimide reaction. As shown in Fig. 6, the pristine GE and LDHs-*g*-POEGMA-GE displayed inhibition zone with 24 and 14 mm of diameter (Fig. 6(a) and (b)), whereas LDHs-*g*-POEGMA was considered without inhibition zone (Fig. 6(c)). The results indicated that the high antibiotic activity of composites has been improved by the presence of GE. The *in-vitro* result demonstrated the effective antibacterial of LDHs-*g*-POEGMA-GE composites.

Conclusions

LDHs-*g*-POEGMA-GE composites were prepared by the RAFT polymerization of OEGMA in the presence of surface-functionalized LDHs followed by the esterification reaction of GE.

The structure of composites was confirmed by using FT-IR, XRD, and TGA. The morphologies of composites were examined by SEM analysis. The LDHs-*g*-POEGMA-GE composites possessed a remarkable antibacterial activity as evidenced by the *in-vitro* test.

Acknowledgment

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