Preparation and Properties of Graphene Oxide Derivative Based Pulmonary Drug Delivery System

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Abstract. GO-F127-RFP composite was prepared by none covalent bond of \(\pi-\pi\)-stacking, and the properties were investigated by Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), field-emission scanning electron microscope (FE-SEM), transmission electron microscope (TEM), drug loading content, antibacterial activity and cytotoxicity assay. The results show that GO-F127-RFP composite is successfully prepared, and the carrier GO-F127 can load more drug RFP than other carriers, GO-F127-RFP composite has the antibacterial ability and GO-F127-RFP can lower the cytotoxicity of RFP, which make it a talent lung targeted system for curing tuberculosis (TB).

Introduction

Partially fueled by the human immunodeficiency virus (HIV) pandemic, tuberculosis (TB) is the second deadyng cause of infectious diseases–associated mortality globally and the leading cause of death among those infected with HIV [1]. In 2013, there were an estimated 11 million prevalent cases, and an estimated 9.0 million incident cases occurred globally [2]. After 2015, TB control will confront extant and emergent challenges, including those directly related to the disease (such as drug resistance and co-infection with HIV) and broader issues (such as the increased prominence of NCDs and instability wrought by mounting income disparities) [3]. Rifampicin (RFP) has the antibacterial activity for mycobacterium tuberculosis bacterium, gram positive bacteria and gram negative bacteria. But adverse drug reactions such as liver toxicity, digestive tract reaction, nervous system disorders and allergic reaction may deteriorate the health-related quality of life, and then may counteract a successful therapy of condition [4]. In consequence, a balance between high therapeutic efficacy and the risk of adverse drug reactions is required, and the development of novel disease-targeted drug strategies is intended for a more effective therapy and demonstrates the potential to address unmet medical needs [5]. Graphene’s large surface area, delocalized \(\pi\) electrons, chemical purity and the possibility of easy functionalization provide opportunities for drug delivery [6].

Herein, we present a drug carrier, graphene oxide derivatives, to load RFP by \(\pi-\pi\)-stacking between them, and the properties were investigated.
Experimental

Preparation of GO-F127-RFP Composites

All chemicals used were of analytical grade and were used as received without any further purification. Deionized water was used in all experiments. Graphene oxide (GO) was prepared from graphite powders through the modified Hummers’ method [7]. The graphene oxide derivative (GO-F127) was prepared using graphene oxide, F-127 and DEC to avoid graphene reunion and promote dispersion in solution at room temperature for 12 h and was reduced by hydrazine hydrate at 40 °C for 12 h [8]. The RFP methanol solution and GO-F127 water solution were mixed and stirred for 12 h. The GO-F127-RFP composite was centrifuged, washed with deionized water for three times and gained by freeze-drying.

Analytical Methods

Fourier transform infrared spectra (FTIR) were recorded with the Perkin-Elmer- Spectrum GXS spectrophotometer using KBr pellet. Powder X-ray diffraction (XRD) patterns were recorded by a X-ray diffractometer (MSAL-XD II) using a Cu Kα radiation microscope. The morphologies of obtained GO and GO-F127 were observed by a PHILIPS, ESEM XL 30, Holland field-emission scanning electron microscope (FE-SEM) at an accelerating voltage of 5 kV and a PHILIPS TECNAI-10 microscope, JEOL JEM-2010 (HR) transmission electron microscope (TEM). The drug load content (LC), drug load efficiency (LE) were conducted by ultraviolet and visible (UV/VIS) spectrophotometer. The antibacterial activity of GO-F127-RFP composite was tested by qualitative method (inhibition zone technology). Cytotoxicity of samples were tested using the MTT assay based on the cellular uptake of MTT and its subsequent reduction in the mitochondria of living cells to dark blue MTT formazan crystals.

Result and Discussion

FTIR Analysis

The Fig. 1 showed the FTIR spectra of GO, GO-F127, RFP and GO-F127-RFP composite. That a large quantities of oxygen-containing functional groups exited in the forms of epoxy, hydroxyl and carboxyl groups on the surface of GO demonstrated the successful synthesis of GO by comparing the GO and graphite spectrogram. The broad absorption at 3200-3400 cm\(^{-1}\) was due to the O-H stretching vibration; the peak at 1630 cm\(^{-1}\) could be assigned to the C=O stretching vibration and the peak at 1059 cm\(^{-1}\) was correspond to C-O stretching vibration [9-11]. The new peaks at 1480 and 1102 cm\(^{-1}\) of GO-F127 and new peaks at 1680 cm\(^{-1}\), 1420 cm\(^{-1}\) of GO-F127-RFP composite indicated the GO-F127 and GO-F127-RFP composite were successfully prepared.

XRD Analysis

The XRD patterns of graphite, graphene oxide, graphene oxide derivative and graphene oxide derivative-RFP composites were shown in Fig. 2 which reveal information on their structural evolution. According to the Prague formula \(2d\sin\theta = n\lambda\), the interlayer spacing will be increase with reduction of \(2\theta\). The XRD spectrum of graphite exhibited a strong characteristic (0 0 2) peak at 26.6 °, and we could calculate the interlayer spacing of graphite is 3.35 Å. After the oxidation and exfoliation process, this peak disappear in the XPD pattern of the sample and a new peak is observed at 11.2 °, corresponding to the lattice of graphene
oxide, which indicates that graphite was converted to graphene oxide [12]. The calculated interlayer spacing of graphene oxide is 7.89 Å, which is much larger than that of graphite. The XRD patterns of graphene oxide derivative and graphene oxide derivative-RFP composites show a broad peak at 7.2° with an interlayer spacing of 10.51 Å and a broad peak at 4.7° with an interlayer spacing of 18.96 Å, respectively, which demonstrates the F127 functionalize the graphene oxide and RPF were loaded by π-π-stacking to make the interlayer spacing lager.

![Figure 1. FT-IR spectra of (A) GO; (B) RFP; (C) GO-F127; (D) GO-F127-RFP.](image1)

![Figure 2. XRD patterns of (A) GO; (B) RFP; (C) GO-F127; (D) GO-F127-RFP.](image2)

**SEM Analysis**

The morphologies of GO and GO-F127-RFP were imaged by FE-SEM and were shown in Fig. 3. The surface of GO was smooth which was corresponding to its lamellar structure, but that of GO-F127-RFP appeared some honeycomb holes for GO layers deformation by strong π-π-stacking.

![Figure 3. SEM images of (A) GO; (B) GO-F127-RFP.](image3)

**TEM Analysis**

The TEM images of GO and GO-F127-RFP were shown in Fig. 4. It can be seen that the pure GO particles showed lamellar structure of the fold and the fold was formed by stacking between GO sheets. But in GO-F127-RFP composite, the F127 and RFP were almost uniformly deposited on the surface of graphene which indicated the GO-F127-RFP composite was prepared successfully.
Figure 4. TEM images of (A) GO and (B) GO-F127-RFP.

**Drug Load Research**

According to the formula, we worked out that the LC was 150.3% and LE was 30.6% that was much more than other carriers [13, 14], which indicated graphene oxide derivative had the potential to be a drug delivery carrier.

**Antibacterial Activity**

The antibacterial activity to attenuated strains BCG of GO-F127-RFP composite was shown in Fig. 4 and Fig. 5. The attenuated strains BCG were trained for three weeks, the colonies decreased with the concentration add of GO-F127-RFP composite, and the minimal inhibitory concentration (MIC) was 40 μg/ml, which showed that this nano-composite had the antibacterial activity.

Figure 5. The impact of different concentration of GO-F127-RFP nutrient medium to BCG bacteria.

Figure 6. Evaluation of MIC of GO-F127-RFP resistant bacteria BCG.

**Cytotoxicity Assay**

The cytotoxicity test on the samples was also carried out. As shown in fig.7, the GO-F127 and dissociative RFP exhibited a serious cytotoxicity to NIH-3T3 within 24 h incubation (the cell viability of NIH-3T3 was reduced to 55% with 500 μg/mL RFP). However, the cell viability of NIH-3T3 was reduced to 62.6% with 500 μg/mL RFP-loaded GO-F127-RFP.
Therefore, the cytotoxicity of GO-F127-RFP was lower than SASP, and the use of GO-F127-RFP would be safer than the direct use of SASP.

Figure 7. Cytotoxicity of GO-F127 and GO-F127-RFP on Hep2 cells.

Conclusion

The GO-F127-RFP composite was prepared by none covalent bond of π-π-stacking. The drug loading content of GO-F127 carrier was 150.3% which was much more than other carriers, and the colonies decreased with the concentration add of GO-F127-RFP composite and the minimal inhibitory concentration (MIC) was 40 μg/ml which showed the antibacterial activity of GO-F127-RFP composite, the cytotoxicity of GO-F127-RFP was lower than SASP, and the use of GO-F127-RFP would be safer than the direct use of SASP, which indicated that the GO-F127 would be a talent drug delivery carrier for curing TB of lung targeted.

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