Construction of an exosome-functionalized graphene oxide based composite bionic smart drug delivery system and its anticancer activity

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Abstract
Graphene oxide has covalently modified by chito oligosaccharides and γ-polyglutamic acid to form GO-CO-γ-PGA, which exhibits excellent performance as a drug delivery carrier, but this carrier did not have the ability to actively target. In this study, the targeting property of breast cancer tumor cell exosomes was exploited to give GO-CO-γ-PGA the ability to target breast tumor cells (MDA-MB-231), and the drug mitoxantrone (MIT) was loaded to finally form EXO-GO-CO-γ-PGA-MIT with an encapsulation efficiency of 73.02%. The pH response of EXO-GO-CO-γ-PGA showed a maximum cumulative release rate of 56.59% (pH 5.0, 120 h) and 6.73% (pH 7.4, 120 h) for MIT at different pH conditions. In vitro cellular assays showed that EXO-GO-CO-γ-PGA-MIT was more potent in killing MDA-MB-231 cells due to its targeting ability and had a significantly higher pro-apoptotic capacity compared to GO-CO-γ-PGA-MIT. The results showed that this bionic nano-intelligent drug delivery system has good drug slow release function and it can increase the local drug concentration of tumor and enhance the pro-apoptotic ability of MIT, so this newly synthesized bionic drug delivery carriers (EXO-GO-CO-γ-PGA-MIT) has potential application in breast cancer treatment.

Supplementary material for this article is available online

Keywords: graphene oxide, exosome, targeted therapy, breast cancer

(Some figures may appear in colour only in the online journal)

1. Introduction
Breast cancer is one of the most common malignant tumors in women, and the incidence and mortality rate of breast cancer has remained high in recent years [1–5]. Surgery, radiotherapy and chemotherapy are the main treatment options for breast cancer [6]. Chemotherapy is the main treatment for breast cancer besides surgery, but chemotherapy drugs usually have strong cytotoxicity, stability and poor selectivity, which can not only kill tumor cells, but also have a killing effect on normal cells, causing adverse reactions [7]. Therefore, the development of safe and efficient tumor treatment modalities is the main goal of future science.

To solve the above problems, nano-delivery systems have emerged, and typical nano-delivery systems include nanoparticles, liposomes, micelles, etc [8]. As a new carbon nanomaterial, graphene oxide has advantages that other nanomaterials do not have, such as the presence of easily modified functional groups, large specific surface area and...
good biocompatibility [9]. The hydrophobic structure of GO is suitable for drug loading through non-covalent bonds, especially π–π stacking and hydrophobic interactions, providing opportunities for drug delivery [10]. Based on these characteristics, graphene oxide is widely used as a drug delivery system in biomedical applications [11]. For example, Wu et al bound ethylenediamine-β-cyclodextrin (EDA-CD) to GO through an amide bond and loaded both the anticancer drug DOX and topotecan (TPT). The results showed that this drug delivery system had better anti-tumor effects than the free drug and the nanoparticles loaded with single drug [12]. Mojtaba et al used chitosan (CS) and methacrylic acid (MAA) modified graphene oxide loaded with adriamycin (DOX) to treat breast tumors and showed promising therapeutic results [13].

Using various oxygen-containing functional groups (such as carboxyl and hydroxyl groups) on the surface of graphene oxide, the GO surface can be functionalized to form a well-targeted and biocompatible anti-tumor drug carrier [14, 15]. Exosomes (Exo) are small vesicles with a lipid bilayer structure, with a particle size of 30–150 nm, produced by the endosomal pathway and secrete into the extracellular space to function [16–18]. As they diffuse not only locally but also systemically through the blood circulation, exosomes encapsulate miRNAs, proteins and lipids, making them an important means of intercellular communication between neighboring cells [19]. Exosomes exhibit effective cellular uptake dependent on their membrane proteins [20–23]. Studies have shown that tumor-derived exosomes have the ability to home in on tumor cells [24]. Integrins on the exosomal membrane of tumor cells may be involved in their specific targeting to tumors, which may be one of the reasons for the homing effect of exosomes [25]. To address the homing property of tumor cell exosomes to parental cells, Yong et al prepared exosome-encapsulated porous silica nanoparticles (DOX @ E-PSINPs) using human hepatocellular carcinoma cells engulfed with DOX, which showed specific targeting ability to hepatocellular carcinoma cells [26]. In this study, we formed EXO-GO-CO-γ-PGA by covalently modifying the nanocarrier GO-CO-γ-PGA with an amino group on the surface of the exosome membrane of breast cancer tumor cells (MDA-MB-231) [27–30], which enables active delivery of the antitumor drug MIT to MDA-MB-231 cells. We have conducted an in-depth study on the drug-controlled release ability, biosafety and anti-tumor mechanism of this nanodelivery system (EXO-GO-CO-γ-PGA-MIT), which provides a new idea for the treatment of breast cancer.

2. Materials and methods

2.1. Isolation of exosomes

Cell culture using DMEM medium (BI, Israel). Exosomes were isolated from the supernatant of MDA-MB-231 (Shanghai Institute of Biochemistry and Cell Biology, China) cell culture by differential ultracentrifugation. Firstly, the supernatant of MDA-MB-231 cell culture supernatant was collected and centrifuged at 4 °C, 1000× g for 10 min, and the supernatant was centrifuged at 4 °C, 10 000× g for 30 min, and the supernatant was extracted; the supernatant from the previous step was transferred to an ultracentrifuge tube, and the supernatant was centrifuged at 4 °C, 100 000× g for 90 min using an ultracentrifuge (Optima XPN-90, BECKMAN COULTER, USA). Centrifuge at 100 000× g for 90 min and discard the supernatant to take the precipitate. The precipitate was resuspended with PBS and centrifuged again at 4 °C for 90 min at 100 000× g. Resuspend the precipitate with a small amount of PBS and proceed directly to the next experiment or transfer to −80 °C for storage.

2.2. Synthesis of EXO-GO-CO-γ-PGA

GO-CO-γ-PGA was synthesized according to the previous method [31], as described in supplementary Files S1 (available online at stacks.iop.org/NANO/33/175101/mmedia). Weighed 10 mg of GO-CO-γ-PGA and dissolved in 10 ml of 20 mM Tris-HCl buffer containing 0.1 M KCl (pH 7.4), sonicated for 10 min, then added cross-linking agent (EDC 424 mg and NHS 636 mg (Sigma-aldrich, USA)), sonicated for 10 min and placed on a shaker at 37 °C, 160 r, for 20 min. Adjusted the mixture to pH 7.4 and then added 40 μg of exosome to the solution. 40 μg of exosomes were added to the solution, placed on a shaker for 10h, washed by centrifugation, dialyzed in ultrapure water, and freeze-dried to obtain the newly synthesized bionic drug delivery carriers. The appropriate drug delivery carriers were weighed and dispersed into pH = 7.4 PBS buffer for subsequent experiments.

2.3. Characterization

The infrared spectra of the samples were determined using a Fourier transform infrared spectrometer (FT-IR) (Nicolet is 5, Thermo Fisher Scientific, USA). The chemical composition of the samples was analyzed using an x-ray photoelectron spectrometer (XPS) (ESCALAB 250Xi, Thermo Fisher Scientific, USA). The UV–visible absorption spectra of the samples were analyzed using a UV–visible spectrophotometer (Evolution 350, Thermo Fisher Scientific, USA). The particle size and zeta potential were examined by a zeta potential analyzer (Zetasizemano, Malvern, UK). The morphology of the samples was observed by transmission electron microscopy (TEM) (JEM-2100 PLUS, JEOL, Japan). The detection of the surface protein integrity of the samples was performed by electrophoresis (Mini-PROTEAN® Tetra, Bio-Rad,USA) for separation and developed using a gel imaging system (Chemi Doc XRS+-, USA).

2.4. Drug loading and release

40 mg of EXO-GO-CO-γ-PGA was dissolved in 40 ml of PBS buffer (pH 7.4), and the mixture was divided equally into 10 parts. Add 4 ml of MIT solution at concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mg ml⁻¹, respectively stir for 24 h at room temperature and avoid light, centrifuge the supernatant and measure the absorbance of the
supernatant by UV spectrophotometer. The drug encapsulation efficiency was calculated from the MIT standard curve (figure S5) with the following equation:

\[
\text{Encapsulation efficiency} \% = \left( \frac{m_{MIT} - m'_{MIT}}{m_{MIT}} \right) \times 100\%,
\]

where \(m_{MIT}\) denotes the mass of initial drug, \(m'_{MIT}\) denotes the mass of residual drug in the solution after loading drug delivery carriers.

5 mg of EXO-GO-CO-\(\gamma\)-PGA was dissolved in 5 ml of PBS buffer (pH 7.4) and sonicated for 10 min. 5 ml of MIT solution at a concentration of 1.8 mg ml\(^{-1}\) was added to the above solution, and the reaction was stirred at room temperature without light for 24 h. The unloaded drug was removed by high-speed centrifugation, and the precipitate was dried using a freeze-dryer to obtain EXO-GO-CO-\(\gamma\)-PGA-MIT. Similarly, GO-CO-\(\gamma\)-PGA-MIT was synthesized using the same conditions.

Different pH buffers were set up in vitro to simulate the in vivo tumor environment for MIT release studies in buffers of different pH. The samples were dissolved in PBS buffer of different pH (pH 7.4, pH 5.0 and pH 1.2) and transferred to dialysis bags, which were placed in the above PBS buffer at 37 °C, respectively. Equal amounts of release solution were collected at regular intervals and replenished with isothermal PBS buffer for 168 h. The absorbance of MIT was measured and the amount of MIT released was calculated.

### 2.5. Cell uptake

MDA-MB-231 cells and BEAS-2B cells were inoculated in 6-well plates, and when the cells grew to about 80%, MIT, GO-CO-\(\gamma\)-PGA-MIT and EXO-GO-CO-\(\gamma\)-PGA-MIT were added, respectively, so that the final drug concentration was 5 \(\mu\)g ml\(^{-1}\) (MIT concentration). After co-incubation for 6 h, wash off excess drug delivery systems that do not enter the cells and the cells were collected for detection by flow cytometry (FACSVerse, BD, USA).

### 2.6. Intracellular imaging

Breast cancer cells and normal cells were inoculated in 6-well plates, and when the cells grew to about 80%, GO-CO-\(\gamma\)-PGA-MIT and EXO-GO-CO-\(\gamma\)-PGA-MIT were added to make the final drug concentration of 5 \(\mu\)g ml\(^{-1}\) (MIT concentration), respectively. After co-incubation for 2 h, the drugs that did not enter the cells were washed away, and the nuclei were stained using DAPI (Thermo Fisher Scientific, USA) and finally observed using a two-photon confocal microscope (LSM 880NLO, Carl Zeiss, Germany).

### 2.7. Cytotoxicity assay

Cells were inoculated in 96-well plates with cells grown to about 80% adding different concentrations (determine the optimal concentration range of the drug by pre-experimentation figure S6) of samples, incubated for a total of 24 h then MTT (Thermo Fisher Scientific, USA) was added to continue incubation for 4 h, the supernatant was removed, and the crystals formed were fully dissolved after adding equal amount of DMSO to each well, and the absorbance value at 570 nm was detected using an enzyme marker (EPOCH2, BioTek, USA) using the following formula to detect cell
OD

viability.

Cell viability(%) = \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{Control}} - OD_{\text{blank}}} \times 100\%,

where $OD_{\text{sample}}$ is the absorbance value of the sample, $OD_{\text{blank}}$ is the absorbance value of the blank group, and $OD_{\text{Control}}$ is the absorbance value of the control group.

### 2.8. Apoptosis assay

Breast cancer cells were inoculated in 6-well plates, and when the cells grew to about 80%, MIT, GO-CO-$\gamma$-PGA-MIT and EXO-GO-CO-$\gamma$-PGA-MIT with different drug concentrations were added, and incubated for a total of 24 h. After washing away the drugs that did not enter the cells, the cells were collected and treated using the apoptosis detection kit, flow cytometry test results.

## 3. Results and discussion

### 3.1. Exosome characterization

Exosomes were isolated from MDA-MB-231 cell culture supernatants using differential ultracentrifugation as described in the above method, and the isolated exosomes were characterized: (1) morphologically by TEM, (2) by size distribution using a particle size analyzer, and (3) by simultaneous detection of marker proteins of exosomes using Western blot. As shown in figure 1(a), the purified exosomes exhibited a typical teato-like round vesicle structure under transmission electron microscopy. The size distribution of exosomes was wide, ranging from 60 nm to 100 nm (figure 1(b)). Exosomes are secreted from cells that carry abundant proteins on the cell membrane surface, including transmembrane and cytoplasmic proteins. Western blot was used to detect the expression of the selective four-transmembrane protein CD63, the cytoplasmic protein TSG101, and Alix, a protein involved in multi-vesicle formation, as well as Calnexin, an endoplasmic reticulum-derived protein used as a control, in order to demonstrate that the extracted vesicles were exosomes [32–37]. As shown in figure 1(c), the isolated exosomes expressed CD69, TSG101 and Alix protein, but Calnexin could not be detected in the exosomes but was detected in normal cells, indicating that the isolated exosomes were pure and uncontaminated. In summary, we were able to isolate exosomes from MDA-MB-231 cell culture supernatant and use them for subsequent experiments.
3.2. Characterization

Fourier infrared spectroscopy (FT-IR), ultraviolet-visible absorption spectroscopy (UV–vis) were used to characterize the structure of the samples. The dispersion of the samples was detected by Zeta potential measurements, their chemical elemental composition was characterized by x-ray photoelectron spectroscopy (XPS), and finally the morphology of the samples was characterized using transmission electron microscopy (TEM). Figure 2(a) shows that the absorption peaks of the infrared spectra of GO are located at 3424 cm\(^{-1}\) and 1733 cm\(^{-1}\), which are due to the O–H stretching vibration and carbonyl stretching of the COOH group in GO; the absorption peak at 1623 cm\(^{-1}\) is attributed to the C=C bond in GO\[^{[38]}\]; the absorption peak at 1070 cm\(^{-1}\) is a C=O stretching vibration peak. FT-IR spectra of GO-CO-\(\gamma\)-PGA shows the characteristic peaks as C=O stretching vibration peak at 1630 cm\(^{-1}\) (amide I belt) and N–H bending vibration peak at 1553 cm\(^{-1}\) (amide II belt). The FT-IR spectra of EXO-GO-CO-\(\gamma\)-PGA after exosome surface modification shows a C=O stretching vibration peak at 1641 cm\(^{-1}\) (amide I belt) and an N–H bending vibration peak at 1553 cm\(^{-1}\) (amide II belt) with a mild increase at the amide band peak. In addition, C=O peak also appeared at 1705 cm\(^{-1}\), which came from ketone carbonyl group on the surface of cell membrane, and P=O stretching vibration peak at 1243 cm\(^{-1}\) indicated that phosphorus element was introduced into the synthesized nano drug delivery carrier, which all these chemical bonds is derived from the membrane of exosome\[^{[38–40]}\]. The above FT-IR results indicate that the exosomes have been modified onto the nanocarriers surface.

When nanocarriers is modified with exosomes, changes in the elemental composition of the material are found. we used XPS spectroscopy to detect the elemental changes (figure 2(b)). As known in the figure after exosome modification EXO-GO-CO-\(\gamma\)-PGA has an increased proportion of nitrogen elements compared to GO-CO-\(\gamma\)-PGA (from 27.7% in GO-CO-\(\gamma\)-PGA to 30.1% in EXO-GO-CO-\(\gamma\)-PGA). Since exosomes contain phosphorus and GO-CO-\(\gamma\)-PGA does not contain phosphorus, the percentage of phosphorus in EXO-GO-CO-\(\gamma\)-PGA after exosome modification reached 2.7%, demonstrating that exosomes have been successfully attached to nanocarriers.

Figure 2(c) shows the UV–vis spectrum of EXO-GO-CO-\(\gamma\)-PGA. The absorption peak at 230 nm is mainly due to the \(\pi-\pi\) leap of the C=C bond in GO\[^{[41]}\]. The exosomes mainly contain proteins, lipids and nucleic acids so that the characteristic peak appeared at 238 nm\[^{[42, 43]}\]. After exosome modification, the newly synthesized bionic drug delivery carriers shows a new peak at 257 nm, and the characteristic peak was shifted to shorter wavelengths. The experimental results indicate that the exosomes have been successfully ligated to form EXO-GO-CO-\(\gamma\)-PGA.

Stability of the newly synthesized bionic drug delivery carriers as relected by Zeta potential. The smaller the dispersed particles, the larger the absolute value of zeta potential (positive or negative), and the more stable the system. On the contrary, the lower the absolute value of zeta potential...
(positive or negative), the more it tends to coalesce or agglomerate [44, 45]. The Zeta potential of the newly synthesized bionic drug delivery carriers at pH 7.4 was $-36.8$ mV, while that of GO-CO-$\gamma$-PGA was $-23.7$ mV. The experimental results indicate that the newly synthesized bionic drug delivery carriers showed good stability, which also indirectly proved the successful synthesis of EXO-GO-CO-$\gamma$-PGA (figure 3(a)).

The morphology of the newly synthesized bionic drug delivery carriers was characterized by TEM, as shown in figure 3(a), after exosome modification, the lamellar surface of GO-CO-$\gamma$-PGA contained plenty of vesiculate structures, while no vesiculate structures were seen on the lamellar surface of the unmodified GO-CO-$\gamma$-PGA. Figure 3(b) As shown by TEM, exosomes have been successfully modified to the surface of GO-CO-$\gamma$-PGA, proving the successful construction of EXO-GO-CO-$\gamma$-PGA.

For the purpose of verifying whether the proteins targeted by exosomes remain intact after exosome modification to the surface of GO-CO-$\gamma$-PGA, SDS-PAGE gel electrophoresis was used to analyze the composition content of exosome proteins before and after modification. As shown in figure 3(c), the exosomes on EXO-GO-CO-$\gamma$-PGA were well preserved compared with the exosome proteins before modification, confirming that the membrane proteins were not lost during the preparation of the newly synthesized bionic drug delivery carriers.

### 3.3. Loading and release of MIT

The newly synthesized bionic drug delivery carriers were loaded with mitoxantrone via $\pi-\pi$ stacking and hydrophobic interaction [46–49]. Drug delivery carriers after drug loading were characterized using Fourier infrared spectroscopy (FTIR) and ultraviolet-visible spectroscopy (UV–vis).

The absorption peaks of the infrared spectra of MIT as shown in figure S1 are located at $1607$ cm$^{-1}$ and $1562$ cm$^{-1}$, which are C–O stretching vibration peaks from the skeletal anthraquinone ring. The absorption peaks of the infrared spectrum of GO-CO-$\gamma$-PGA-MIT showed its characteristic peaks at $1641$ cm$^{-1}$, $1607$ cm$^{-1}$, $1070$ cm$^{-1}$, where the appearance of C–O stretching vibration peak at $1641$ cm$^{-1}$ (amide I band) and the C–O stretching vibration peak at $1070$ cm$^{-1}$ were from the carrier GO-CO-$\gamma$-PGA, while the C–O stretching vibration peak at $1609$ cm$^{-1}$ C–O absorption peak at $1609$ cm$^{-1}$ was from MIT. The infrared spectra of EXO-GO-CO-$\gamma$-PGA-MIT showed (figure S2) that its absorption peaks were $1641$ cm$^{-1}$, $1609$ cm$^{-1}$, $1244$ cm$^{-1}$, where the appearance of C–O stretching vibration peak at $1641$ cm$^{-1}$ (amide I band) and the P=O stretching vibration peak at $1244$ cm$^{-1}$ were from the carrier EXO-GO-CO-$\gamma$-PGA, while the skeletal anthraquinone ring absorption peak at $1609$ cm$^{-1}$ was from MIT. The above FTIR results show that MIT was successfully loaded to GO-CO-$\gamma$-PGA and EXO-GO-CO-$\gamma$-PGA.

As shown in figures S3 and S4 showing strong UV absorption peaks at $237$ nm and $610$ nm for MIT, and strong UV absorption peaks at $610$ nm for GO-CO-$\gamma$-PGA-MIT and EXO-GO-CO-$\gamma$-PGA-MIT, which further demonstrate that MIT has been loaded onto the drug delivery carriers.

In order to determine the loading of MIT by the newly synthesized bionic drug delivery carriers, we conducted loading experiments using different concentrations of MIT with equal amounts of EXO-GO-CO-$\gamma$-PGA. As shown in figure 4(a), the drug loading capacity of EXO-GO-CO-$\gamma$-PGA increased gradually with the increase of MIT concentration and the maximum drug loading capacity was larger than before the modification. The drug concentration was $1.8$ mg ml$^{-1}$ when the carrier reached saturation, and the maximum drug encapsulation efficiency was changed from 62.43% to 73.02% after exosome modification.

The main feature of the tumor physiological microenvironment is the high metabolic rate which causes many areas within the tumor to be temporarily or chronically weakly acidic [50–52]. So we simulated different internal environments in vitro to verify the controlled and slow release of EXO-GO-CO-$\gamma$-PGA on MIT under different internal environments. We measured the drug release at pH 1.2, 5.0 and 7.4. As shown in figure 4(b), both drug delivery systems are capable of showing a rapid release trend within $24$ h and a slow release trend after $24$ h. The maximum cumulative drug...
release is achieved at 120 h for both delivery systems. The cumulative drug release rates were higher when pH = 1.2 than at pH 5.0 and 7.4, indicating that both drug delivery systems have better pH sensitivity. The cumulative release rate of the exosome-modified drug delivery vehicle reached a maximum of 64.25% (pH 1.2), 56.32% (pH 5.0) and 6.59% (pH 7.4), which was higher than that of the before retouching at 61.25% (pH 1.2), 51.12% (pH 5.0) and 5.52% (pH 7.4).

Tumor microenvironment pH is close to 5.0, while the normal physiological environment pH is around 7.4 [50, 51]. The cumulative release of MIT from the exosome-modified drug delivery carriers was significantly higher under acidic conditions than under neutral conditions, indicating that EXO-CO-γ-PGA-MIT is more stable in normal blood transport and can release more drug at the tumor site thus reducing toxicity to normal cells. Above values indicate that EXO-GO-CO-γ-PGA has a large drug encapsulation efficiency, pH sensitivity and better controlled and slow release of the drug.

3.4. Intracellular imaging

Figure 5 shows the laser confocal images of GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA-MIT after 2 h treatment of normal and breast cancer cells, respectively. As shown in figure 5, GO-CO-γ-PGA-MIT was mainly distributed in the cytoplasm of Beas-2B cells with a small amount in the nucleus, while EXO-GO-CO-γ-PGA-MIT was mainly distributed in the nucleus of Beas-2B cells with a small amount in the cytoplasm, indicating that the ability of EXO-GO-CO-γ-PGA to transport drugs was higher than that of GO-CO-γ-PGA. For MDA-MB-231 cells, GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA-MIT were both distributed in the nucleus and a small amount in the cytoplasm. However, EXO-GO-CO-γ-PGA-MIT was abundantly and concentrically distributed in the nucleus and cytoplasm, indicating that EXO-GO-CO-γ-PGA-MIT could increase drug entry into MDA-MB-231 cells. The above results show that
Figure 6. Flow cytometry analyses of MIT uptake in (a) Beas-2B cells and (b) MDA-MB-231 cells treated with MIT, GO-CO-\(\gamma\)-PGA-MIT and EXO-GO-CO-\(\gamma\)-PGA-MIT.

Figure 7. (a) The effect of different concentration of unloaded EXO-GO-CO-\(\gamma\)-PGA against MDA-MB-231 and Beas-2B cells viability. (b) The effect of different concentration of MIT, GO-CO-\(\gamma\)-PGA-MIT and EXO-GO-CO-\(\gamma\)-PGA-MIT against MDA-MB-231 cells viability. (c) The effect of different concentration of MIT, GO-CO-\(\gamma\)-PGA-MIT and EXO-GO-CO-\(\gamma\)-PGA-MIT against Beas-2B cells viability.
EXO-GO-CO-γ-PGA can specifically target MDA-MB-231 cells and enable more drugs to enter tumor cells.

3.5. Cellular uptake

To quantify drug entry into cells, flow cytometry was used to detect the intracellular drug fluorescence intensity. Figure 6(a) shows the fluorescence intensities of MIT, GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA-MIT in normal cells (Beas-2B cells) of 9326, 9406 and 10065, respectively. Figure 6(b) shows the fluorescence intensities of MIT, GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA -MIT in breast cancer cells (MDA-MB-231 cells) with fluorescence intensities of 10290, 10940, and 11381, respectively. The results show that exosome-modified nanocarriers can deliver more drugs to cells due to the targeting nature of exosomes. Intracellular imaging and cellular uptake experiments demonstrate that EXO-GO-CO-γ-PGA-MIT accumulated more in tumor cells than GO-CO-γ-PGA-MIT at the same drug concentration, resulting in better therapeutic efficacy at equal.

3.6. Cytotoxicity assay

MTT assay was used to detect the effect of samples on cell viability. According to the figure 7(a), the effects of different concentrations of EXO-GO-CO-γ-PGA on the cell viability of normal cells (Beas-2B) and breast tumor cells (MDA-MB-231) can be shown that the survival rate of both normal and tumor cells was above 80% even when the carrier concentration reached 200 μg ml⁻¹, which indicates that the newly synthesized bionic drug delivery carrier has a good biosafety and can be used as a drug transport carrier.

Figure 7(b) shows the cell survival rates of MIT, GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA-MIT acting on breast tumor cells (MDA-MB-231), respectively. As shown in the figure, the cytotoxicity of different drugs on MDA-MB-231 cells gradually increased with the increase of sample concentration in a dose-dependent manner. The exosome-modified drug delivery system showed higher tumor suppression effect than that before modification. The IC50 values of MIT, GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA-MIT for MDA-MB-231 cells were 6.37 μg ml⁻¹, 4.57 μg ml⁻¹ and 3.33 μg ml⁻¹, respectively. The percentage of complete cell survival is shown in table S1. The exosome-modified drug delivery system showed higher tumor suppression effect than that before modification, which was attributed to the targeted nature of the exosomes on the surface of EXO-GO-CO-γ-PGA-MIT to precisely target tumor cells, thus showing better anti-tumor effects.
To further validate the targeting of EXO-GO-CO-γ-PGA-MIT, we performed its MTT assay on Beas-2B cells (figure 7(c)). By comparing the experimental results in figures 7(b), (c), it was found that MIT, GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA-MIT were more cytotoxicity to MDA-MB-231 cells than to Beas-2B cells. The IC50 values of MIT, GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA-MIT for Beas-2B cells were 5.19 μg ml−1, 4.53 μg ml−1 and 4.69 μg ml−1, respectively. The cytotoxicity of GO-CO-γ-PGA-MIT and EXO-GO-CO-γ-PGA-MIT was not significantly different in Beas-2B cells, while for MDA-MB-231 cells, the cytotoxicity of the exosome-modified drug delivery system was significantly higher than that of the unmodified drug delivery system. The experimental results showed that EXO-GO-CO-γ-PGA-MIT has better targeting and higher cytotoxicity to MDA-MB-231 cells.

3.7. In vitro anti-tumor effect

Apoptosis test was performed by flow cytometry. Different concentrations of the samples acted on MDA-MB-231 cells. The apoptosis rate of the cells was detected using the apoptosis assay kit to further verify the anti-tumor effects of the samples. When the MIT concentration was 1.67, 3.33, 6.66 and 9.99 μg ml−1, the apoptosis rates of the MIT group shown in figure 8(a) were 6.33%, 8.97%, 10.19% and 12.64%, respectively. The apoptosis rates of GO-CO-γ-PGA-MIT group shown in figure 8(b) were 11.52%, 13.01%, 16.27%, and 19.03%, respectively. The apoptosis rates from the EXO-GO-CO-γ-PGA-MIT group shown in figure 8(c) were 13.01%, 18.81%, 26.09%, and 39.9%. The results showed that the drug delivery system before and after exosome modification could promote apoptosis and the pro-apoptotic effect of EXO-GO-CO-γ-PGA-MIT was higher than that of GO-CO-γ-PGA-MIT, which were both more effective than MIT.

4. Conclusion

Our study aims to generate synergistic effects by combining the targeting properties of exosomes with the excellent drug delivery vehicle GO-CO-γ-PGA for the precise treatment of breast cancer. In the study, we attached exosomes to the surface of GO-CO-γ-PGA and confirmed the formation of EXO-GO-CO-γ-PGA by FT-IR, XPS, UV and TEM. Compared with GO-CO-γ-PGA, the newly synthesized bionic drug delivery carriers exhibited higher drug loading, better pH response and more excellent biocompatibility. However, the in vivo effect on breast cancer has not been investigated, and the mechanism of the effect of EXO-GO-CO-γ-PGA on breast tumors in vivo can be explored in the future. In conclusion, this bionic nano-intelligent drug delivery system has potential value in anti-breast cancer.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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