# Effect of Vitamin B12-conjugated Nanocomposite on Drug-resistant Gastric Cancer Cells

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**Sericin nanomicelles (Sericin-PBLG) have the properties of efficiently and stably encapsulating hydrophobic chemotherapeutics in aqueous solution. The vitamin B12 (VB12) modified amphiphilic chitosan derivative can improve the delivery effect of oral drugs and has a targeting function. In this study, a new type of sericin nanomedicine (VB12-sericin-PBLG-PTX) loaded with paclitaxel was successfully synthesized. The particle size of VB12-sericin-PBLG-PTX nanomicelles is 121.5 ±1.6 nm. The nanomicelles can reduce the activity of gastric carcinoma cells and gastric carcinoma drug-resistant cells, and increase the apoptosis rate of gastric carcinoma cells and gastric carcinoma drug-resistant cells, and achieve a good anti-tumor effect.**

**Key words: Gastric carcinoma; Vitamin B12; Sericin; nanomicelles**

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## INTRODUCTION

Gastric carcinoma is the most common malignant tumor of the digestive tract. Since there are no specific changes in early gastric carcinoma, most patients are already in the advanced stage when they are diagnosed. The rapid development of surgery, adjuvant radiotherapy and chemotherapy has improved the survival time of gastric carcinoma patients to a certain extent. However, due to chemotherapy resistance and other reasons, the 5-year survival rate of gastric carcinoma patients is only 5% to 15% [1-5]. Gastric carcinoma treatment includes total gastric resection and postoperative systemic intravenous chemotherapy. The scope of surgical resection and standards for lymph node dissection have been basically finalized. However, the postoperative intravenous chemotherapy mode for this type of advanced gastric carcinoma still has significant limitations, mainly due to tumor resistance and lack of tumor-specific targeting of chemotherapeutics, which cannot automatically and efficiently enter the cancer focus [6-8]. Therefore, for patients who are not sensitive to gastric carcinoma chemotherapy drugs, research and development of drugs with (tumor targeting, absorbable for gastric carcinoma microenvironment, reversible transformation therapy resistance/insensitivity) can provide patients with more effective, low toxicity, comfortable and economical comprehensive treatment plan for gastric carcinoma, thereby maximizing the improvement of the prognosis of gastric carcinoma patients. Nano drug delivery technology is a research hotspot for optimizing drug performance in recent years. Based on the modifiable principle of nanocarriers, targeted and multifunctional nano drugs can be designed to achieve smart drug delivery [9].

Sericin is an emerging material in the field of tissue engineering materials and biomedicine in recent years [10]. However, there are few reports on the use of sericin polymers modified by hydrophobic polypeptide segments as drug carriers.In this study, amphiphilic sericin nanomicelles (Sericin-PBLG) were synthesized. The nano micelles not only have the characteristics of excellent biocompatibility,

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biodegradability and easy chemical modification, but also have a protein-like structure that interacts with biological organisms. Therefore, the polymer micelle formed by its solution self-assembly is expected to be used as a kind of better performance biomimetic drug carrier [11]. Paclitaxel (PTX) is an anti-microtubule drug that promotes tubulin polymerization and inhibits depolymerization, keeps tubulin stable, and inhibits cell mitosis. It is currently the most widely used anti-cancer drug [12]. However, PTX is poorly water-soluble and easily causes systemic side effects, such as allergic reactions, bone marrow suppression, neurotoxicity, cardiovascular toxicity, gastrointestinal reactions, and drug resistance [13]. Based on the shortcomings of PTX, this study used the properties of Sericin-PBLG to efficiently and stably encapsulate hydrophobic chemotherapeutics in aqueous solutions to synthesize the nanocomposite Sericin-PBLG-PTX and explore its anti-tumor effect.

However, Sericin-PBLG-PTX lacks the function of actively targeting and accumulating gastric carcinoma lesions. At present, in response to this problem, many researchers couple nano-drugs to tumor targeting probes (such as folic acid) to achieve precise drug delivery to cancer lesions. Treatment [14]. Folic acid, also called vitamin B9, is a water-soluble vitamin. By consulting related literature, it is found that vitamin B12 (VB12) modified amphiphilic chitosan derivatives can improve the delivery effect of oral drugs and have a targeting function [15]. VB12 is an essential vitamin involved in many important links of human cell metabolism, nucleic acid and protein synthesis [16]. The absorption of VB12 in cancer tissues is higher than that of normal tissues [17]. The probes of nuclides and positron derivatives based on VB12 also have broad prospects in tumor imaging and treatment [18]. Therefore, in this study, by constructing fluorescently labeled VB12 probes, high-efficiency accumulation of VB12 can be seen in tumor cells. This phenomenon suggests that VB12 has gastric carcinoma targeting.

Therefore, this article uses VB12 to have the characteristics of active uptake and targeting by gastric carcinoma to synthesize VB12-binding sericin nanocarriers (VB12-sericin-PBLG) to efficiently and stably package the hydrophobic chemotherapeutic PTX in an aqueous solution to explore its resistance to gastric carcinoma cells effect.

## MATERIALS AND METHODS

### Synthesis of VB12-sericin-PBLG Derivatives

Synthesis of Sericin: Silk cocoons were placed in a $Na_2CO_3$ (0.02 M) solution and boiled for 30 min to remove insoluble substances. Subsequently, the supernatant solution after removing the insoluble matter was poured into a cellulose dialysis membrane (with a molecular weight cut-off range of 8-14 kDa), and the cellulose dialysis membrane was placed in deionized water for 3 days of dialysis treatment. Centrifuge at 10,000 rpm for 10 min to remove agglomerates. Finally, the sericin powder was obtained by freeze-drying and stored at 4°C under dry conditions.

Synthesis of Y-benzyl-L-glutamic acid N-carboxylic acid anhydride (BLG-NCA): 5g of L-glutamic acid Y-benzyl ester was completely dissolved in dry tetrahydrofuran (THF). Subsequently, 3.5 g of triphosgene was added, and the reaction was carried out at 55°C and 100% nitrogen for 1 hour. Remove the solvent in a vacuum environment. The oily precipitate was dissolved in 100 mL of ethyl acetate and washed 3 times with saturated NaHCO3 solution. The organic phase was collected and dried with anhydrous MgSCU. After removing the solvent in a vacuum environment, BLG-NCA was obtained.

The hydrophobic sericin hydrophobic peptide Y-benzyl-L-glutamate (PBLG) synthesizes nanomicelles (Sericin-PBLG) through a ring-opening reaction: in a 100% nitrogen environment, 0.5g of sericin powder was dissolved at 50° C in dry DMSO, then cool to 37°C. 0.2g of BLG-NCA powder was added to the DMSO solution, and stir continuously for 72 h at 25°C. The solution was poured into ionized water for 3 days dialysis to remove organic solvents, and freeze-dried to obtain a crude product. The crude product was dispersed in 20 mL of dimethylformamide (DMF), stirred for 30 minutes, and the PBLG homopolymer was removed by centrifugation. The precipitate was dissolved in DMSO, dialyzed again to remove organic solvents and other impurities, and freeze-dried to obtain pure sericin-PBLG.

Synthesis of VB12-sericin-PBLG: 600 mg of VB12 (0.04 mmol) was dissolved in 10 mL of dry DMSO, and 82 mg of N,N-Carbonyldiimidazole (CDI, 0.5 mmol) was added. The mixture was reacted under dry nitrogen for 4 h, and then 1 mL of pure water was added to quench the unreacted CDI. 20 mL containing 0.3g DMSO was added to sericin-PBLG, and the mixture was stirred at 25°C for 24h. The compound was poured into ionized water for dialysis and freeze-dried to obtain VB12-sericin-PBLG (yield: 9%).

### Synthesis of VB12-sericin-PBLG-PTX Nanomicelles

VB12-sericin-PBLG micelles were prepared by dialysis. Dissolve 10 mg of VB12-sericin-PBLG

polymer in 1 mL DMSO, then put it into a dialysis bag (molecular weight cut-off [MWCO] 3500Da) and dialyze with distilled water.

Prepare PTX-loaded nanomicelles. Before dialysis, dissolve 1 mg PTX and 20 mg VB12-sericin-PBLG in DMSO and mix them thoroughly. Finally, a 0.45 μm filter was used to remove unencapsulated PTX.

### Characterization of Nanomicelles

The hydrogen NMR spectra of VB12, sericin, sericin-PBLG, and VB12-sericin-PBLG were analyzed by Varian INOVA 500NB NMR spectrometer (500MHz). The VB12 content in VB12-sericin-PBLG polymer was analyzed by ultraviolet absorption spectrometer (PerkinElmer UV7500). In addition, the principle of dynamic light scattering is used to measure the particle size of nanomicelles, and the surface potential of nanomicelles is detected by the Zeta PALS system. A scanning electron microscope (SEM) was used to photograph and analyze the nano micelles.

### In Vitro Release of Nanomicelles

30mL PBS release medium (pH7.4, 0.1M, 1mmol 1-1 sodium salicylate) was prepared. VB12-sericin-PBLG-PTX was placed in a dialysis bag and soaked in PBS solution. Finally, place it on a shaker at 50 rpm. 3.0 mL release medium was taken out at the specified time interval and stored in a centrifuge tube for testing. 20 mg of freeze-dried VB12-sericin-PBLG-PTX nanoparticles were dissolved in 10 mL of acetonitrile and sonicated for 30 minutes. Then the solution was filtered with a 0.45 μm syringe filter, and the content of PTX was detected by high performance liquid chromatography (Waters 1525).

### CCK-8 Assay Was Used to Detect The Activity of Cells

Human gastric carcinoma BGC-823 cells were purchased from ATCC, and a paclitaxel-resistant cell line (BGC-823/DR) of human gastric carcinoma BGC-823 cells was constructed. Gastric carcinoma cells and drug-resistant cells in the logarithmic growth phase were taken out separately, digested with 0.25% trypsin and pipetted into a single cell suspension. The cells were seeded on a 96-well plate at 5000 cells/well. Each well was added 100 μL of cell culture medium containing 10% FBS. The 96-well plate was placed in an incubator (37°C, 5% $CO_2$) and incubated for 24 hours. Sericin-PBLG, VB12-sericin-PBLG, PTX, and VB12-sericin-PBLG-PTX were used to treat the two groups of cells. After 48 hours of incubation, aspirate the medium in the 96-well plate and add 100 μL of medium containing 10% CCK-8 solution. Place the 96-well plate in the incubator and incubate for 2 hours in the dark. Measure the absorbance of each measuring well at 450 nm with MR-96A microplate reader.

### Apoptosis of Gastric Carcinoma Cells Was Analyzed by Flow Cytometry

Gastric carcinoma cells and drug-resistant cells in the logarithmic growth phase were digested with 0.25% trypsin and pipetted into a single cell suspension. Cells were seeded on a 6-well plate at a cell volume of 10,000 cells/well, and 2000 μL of cell culture medium containing 10% FBS was added to each well. The 6-well plate was placed in an incubator (37°C, 5% $CO_2$) and incubated for 24 hours. Sericin-PBLG, VB12-sericin-PBLG, PTX, and VB12-sericin-PBLG-PTX were used to treat the two groups of cells. After the cells were incubated in an incubator (37°C, 5% $CO_2$) for 48 hours, the cells were trypsinized and resuspended. 50,000-100,000 cells were taken out for centrifugation (1000 r/min, 3min), the supernatant was discarded and 100 μL of AnnexinV-FITC was added to resuspend the cells, and mix gently. Incubate in the dark for 10 min and then centrifuge the cells again (1,000 g/min, 5 min). The supernatant was discarded and 400 μL of AnnexinV-FITC was added to resuspend the cells, and gently pipetted to mix. Finally, 5 μL of propidium iodide staining solution was added to each tube of cell samples, mixed gently, and the apoptosis rate was measured on a flow cytometer.

### Statistical Method

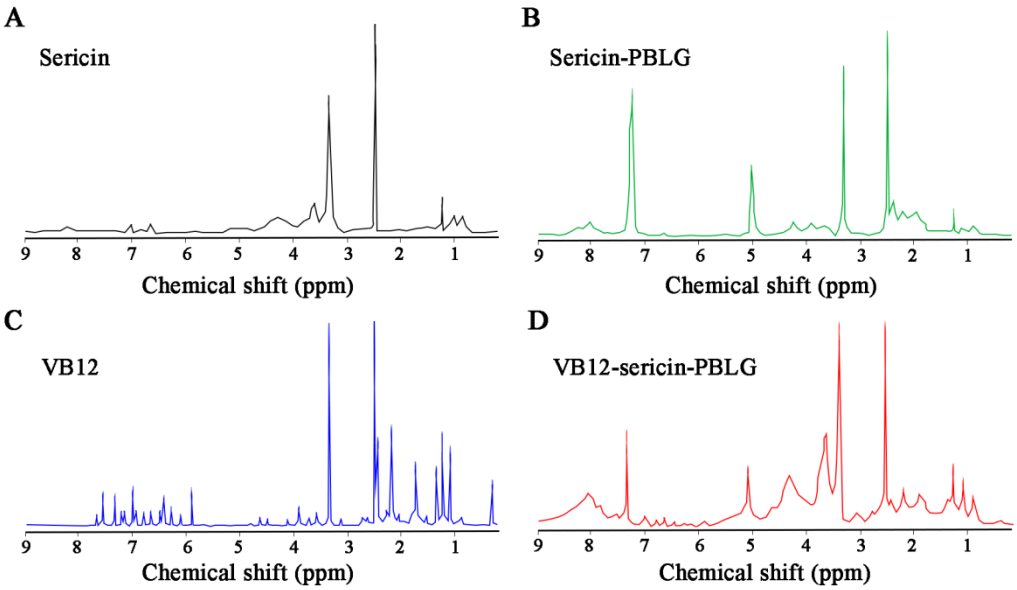
All experiments in this study were repeated at least 3 times. SPSS 24.0 and GraphPad Prism 8 software were used to analyze the experimental data. The comparison of measurement data between the two groups was statistically analyzed by t-test or analysis of variance, and $P < 0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

### The 1H NMR Spectrum of VB12-sericin-PBLG

Compared with sericin (Figure 1A), sericin-PBLG (Figure 1B) can see new special peaks at 4.95 ppm, and 7.30 ppm, which are consistent with the benzene ring protons and benzyl protons of PBLG. In addition, compared with sericin-PBLG, VB12-sericin-PBLG (Figure 1D) has new signal peaks at 6.0-7.0 ppm and 1.5-2.5 ppm. The special peak here is consistent with the signal peak of VB12 (Figure 1C). It is suggested that VB12 is coupled to the sericin-PBLG skeleton.

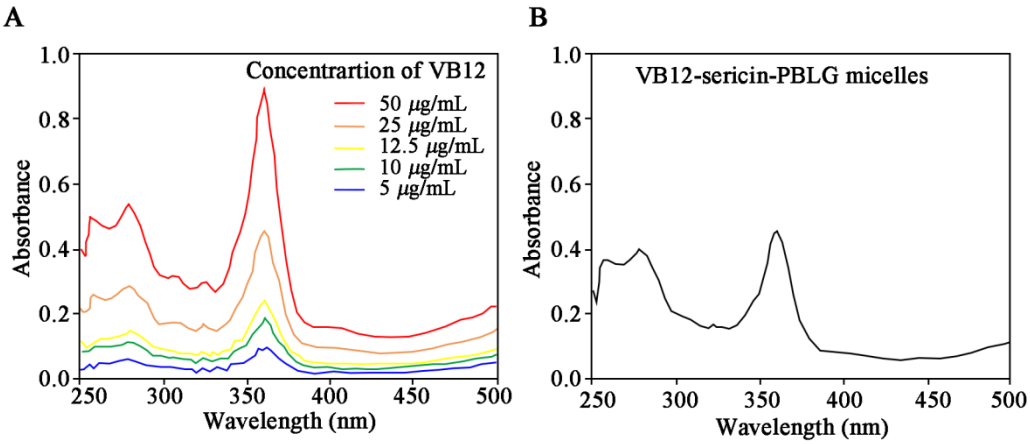
Figure 1.
1H NMR spectrum of sericin (A), sericin-PBLG (B), VB12 (C), and VB12-sericin-PBLG (D).




## Ultraviolet(UV) Absorption Spectrum of VB12-sericin-PBLG

We used UV absorption spectrum to determine the concentration of VB12 in VB12-sericin-PBLG. First, a calibration curve was established based on the UV absorption spectra of VB12 at different concentrations (Figure 2A). Calculation formula: A = E×C×L; "A" is the absorbance of the solution; "E" is the absorbance coefficient, the unit is L/(mol*cm); "C" is the concentration of the solution, the unit is mol/L; "L" is the absorption path, The unit is cm). According to the absorbance of VB12-sericin-PBLG nanomicelles dissolved in DMSO at 365 nm of 0.445 (Figure 2B), we can calculate: C=A/(E×L)= 0.45/(0.018×1) = 25, that is, the content of VB12 in 1 mL of VB12-sericin-PBLG nanomicelles is 25 μg.

Figure 2.
(A) UV absorption spectra of VB12 at different concentrations. (B) UV absorption spectrum of VB12-sericin-PBLG nanomicelles.




## Particle Size and Zeta Potential of VB12-sericin-PBLG

The particle size of sericin-PBLG micelles is 103.1±1.5 nm, which is consistent with the particle size of the drug carrier. The size of VB12-sericin-PBLG nanomicelles after coupling with VB12 is 115.6 ± 1.8 nm. After loading PTX drugs, the particle size of the VB12-sericin-PBLG-PTX nanomicelles is as large as 121.5 ± 1.6 nm (see Table 1). At the same time, the Zeta potential of VB12-sericin-PBLG and VB12-sericin-PBLG-PTX is -25.0 mV, indicating that the micelle surface is negatively charged.

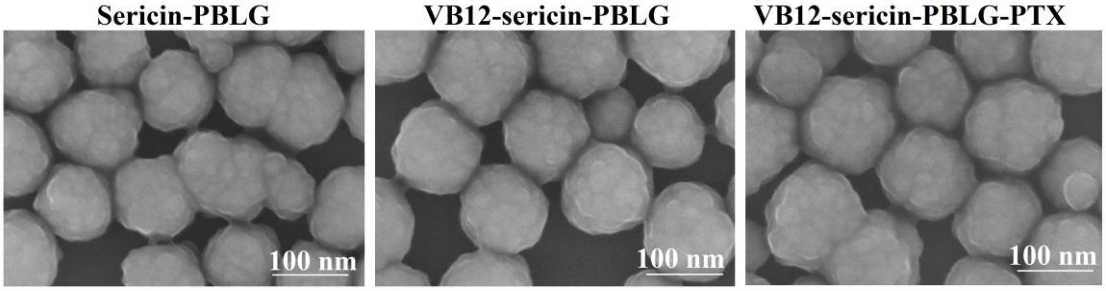
Table 1.
Particle size and Zeta potential of VB12-sericin-PBLG

| | Partical size (nm) | Polymer dispersity index | Zeta potential (mV) |
|---|---|---|---|
| Sericin-PBLG | 103.1±1.5 | 0.26±0.01 | -27.38±1.88 |
| VB12-sericin-PBLG | 115.6±1.8 | 0.25±0.01 | -25.27±1.05 |
| VB12-sericin-PBLG-PTX | 121.5±2.1 | 0.25±0.01 | -24.83±1.22 |

## Transmission Electron Microscope Image of VB12-sericin-PBLG

Under the scanning electron microscope (Figure 3), sericin-PBLG, VB12-sericin-PBLG, and VB12-sericin-PBLG-PTX nanomicelles are spherical, and the particle size is consistent with the result of the hydrated particle size.

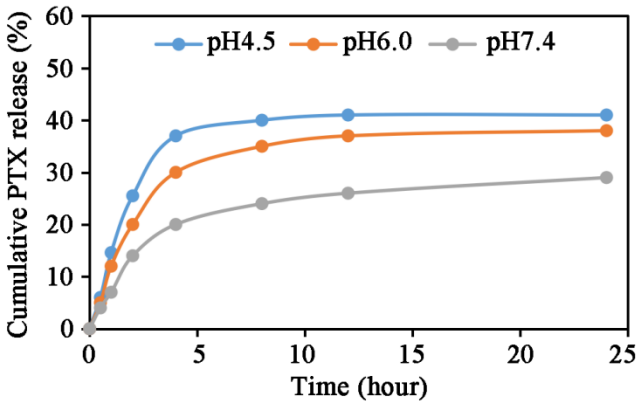
Figure 3.
Transmission electron microscope image




## Dissolution Curve of VB12-sericin-PBLG-PTX

Because the pH values of plasma, early tumor endosomes and lysosomes are 7.4, 6.0, 4.5. Therefore, we set up different pH buffers to monitor the in vitro release of PTX. As shown in Figure 4: In the 24-hour drug release cycle, the release rate of PTX under pH (4.5 and 6.0) conditions is faster than that under pH 7.4 conditions.

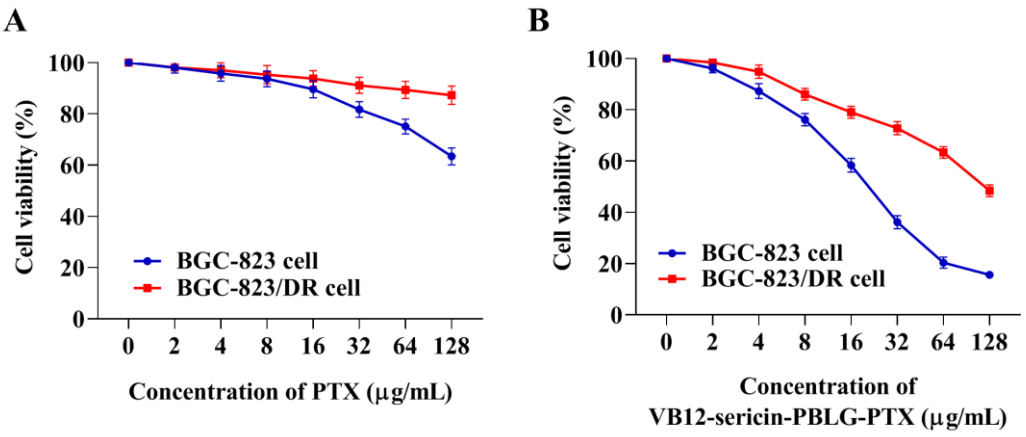
Figure 4.
In vitro dissolution curve of nanomicelles.




## Effect of VB12-sericin-PBLG-PTX on the Activity of Gastric Carcinoma Cells

Compared with the same concentration of PTX, the cell viability of BGC-823 cells and BGC-823/DR cells was significantly reduced under the treatment of VB12-sericin-PBLG-PTX (as shown in Figure 5). This suggests that VB12-sericin-PBLG-PTX can not only target gastric carcinoma cells, but also increase the chemotherapy sensitivity of drug-resistant cells to PTX and reduce the survival rate of drug-resistant cells.

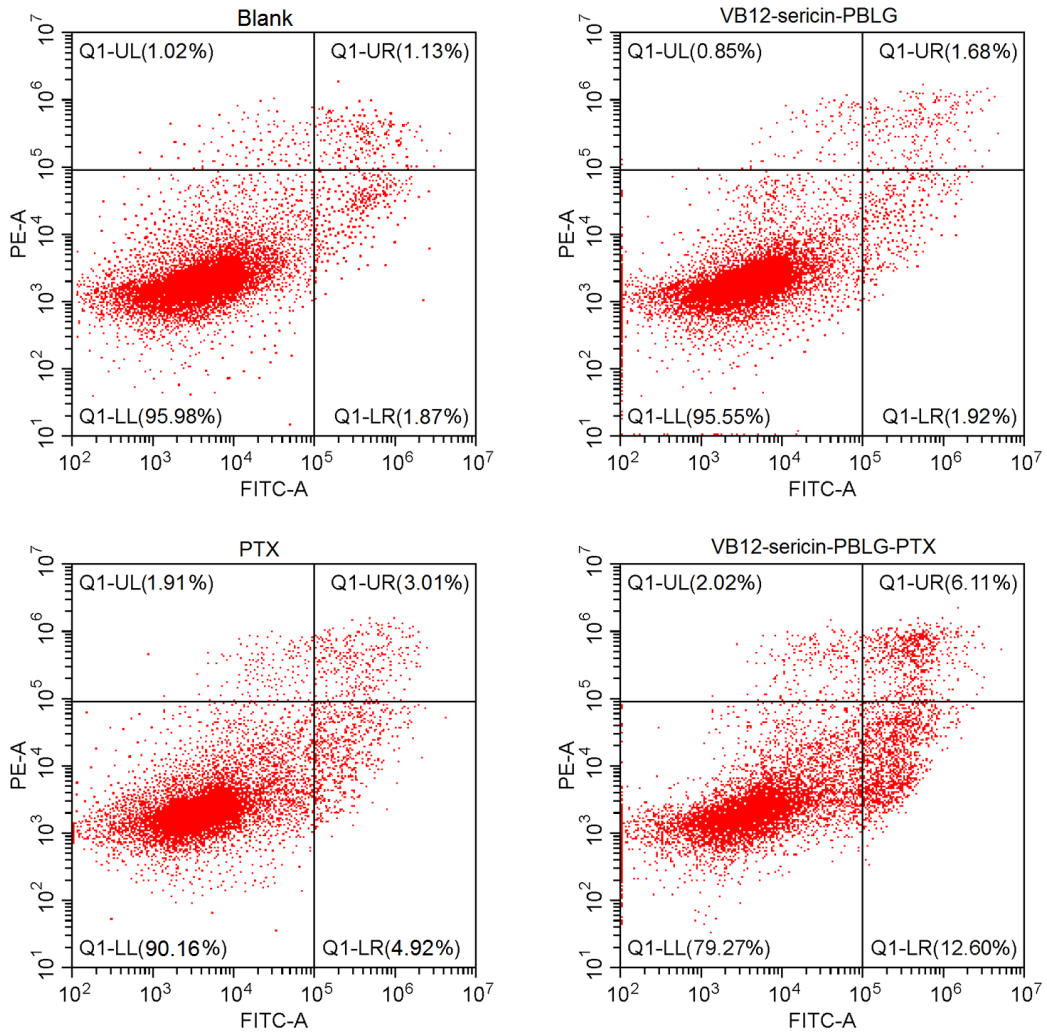
Figure 5.
(A) Activity change curve of different concentrations of PTX on BGC-823 cells. (B) Activity change curve of different concentrations of VB12-sericin-PBLG-PTX on BGC-823 cells




## Effect of VB12-sericin-PBLG-PTX on Apoptosis of BGC-823/DR Cells

The apoptosis rate of the VB12-sericin-PBLG-PTX group (18.71%) was significantly higher than that of the PTX group (7.93%) (as shown in Figure 6). This suggests that VB12-sericin-PBLG-PTX can increase the chemotherapeutic sensitivity of cells to PTX and increase the rate of apoptosis.

**Figure 6.**
**BGC-823/DR cell apoptosis.**




## Discussion

PTX can effectively inhibit spindle function and cause tumor cell death. It is currently the first-line drug for the treatment of gastric carcinoma [19,20]. The early stage of PTX drug use showed strong anti-tumor effects, but with the widespread use of drugs, drug resistance began to appear, and the large amount of drug efflux severely weakened the anti-tumor effect of PTX [21]. At the same time, because PTX is a hydrophobic drug that it cannot enter the tumor site efficiently, coupled with severe cytotoxic side effects, it greatly limits the clinical use of PTX [22-24]. Therefore, it is very necessary to explore and develop new drugs with strong anti-tumor effects. In recent years, nano-drug delivery technology has made breakthrough progress and has unique advantages in anti-cancer chemotherapeutics [25, 26]. The new nano-medicine has a large drug load, and by enhancing the permeability and retention of the drug, enough chemotherapeutic drugs can enter the site of the cancer. In addition, nano-drugs can further enhance the targeting of nano-drugs and exert effective anti-tumor effects by coupling small molecule compounds (such as folic acid) that can specifically bind to molecules on the surface of cancer cells.

Sericin is a natural viscous protein extracted from cocoons, and it has been widely used in many fields such as tissue engineering and biomedicine [29,30]. So far, due to its good biocompatibility and biodegradability, sericin nanocarriers used for drug and gene delivery, including sericin poly(ethylene glycol) nanoparticles, etc. [31]. However, the sericin carrier also has limitations and lacks the ability to efficiently target gastric carcinoma tissues. As we all know, vitamins are essential for the metabolism of all cells. Among them, vitamin B12 is essential for cell proliferation, especially in rapidly expanding and dividing tumor tissues for VB12 [32]. Studies have shown that vitamin B12 has

high water solubility, and its related receptor (transcobalamin II receptor) has a high expression level in tumor tissues [33,34]. Therefore, vitamin B12 has broad application prospects in the diagnosis and treatment of tumors in vivo. New sericin nanoparticles based on VB12 tumor targeting are very valuable for cancer treatment. In this study, we proved through the hydrogen NMR spectrum that we successfully synthesized VB12-modified sericin-PBLG micelles. By measuring the particle size and potential of the nanomicelles, it is shown that VB12-sericin-PBLG-PTX conforms to the particle size of the drug carrier. Through in vitro dissolution experiments, it is proved that VB12-sericin-PBLG-PTX dissolves faster under acidic conditions. Therefore, VB12-sericin-PBLG-PTX, as a new type of nano-drug, has unique advantages in the treatment of gastric carcinoma.

PTX drugs are hydrophobic and easily bind to other plasma proteins in the blood circulation, thereby reducing the concentration of the drug in tumor tissues and reducing its lethality to tumor cells [35]. However, there are reports that the nano-drug carrier system can improve the water solubility of PTX drugs, and mediate the efficient entry of PTX drugs into tumor cells and kill tumor cells [36,37]. In this study, we found that VB12-sericin-PBLG-PTX can significantly reduce the activity of gastric carcinoma cells and gastric carcinoma drug-resistant cells. Compared with PTX, VB12-sericin-PBLG-PTX can significantly induce more drug-resistant cells to undergo apoptosis. However, the specific anti-tumor mechanism of VB12-sericin-PBLG-PTX is still not very clear and needs further study. Among them, the mitochondrial-related pathway is the most common type of apoptosis. When the cells of the body are subjected to external stimulation, the first key change that occurs when the cell undergoes apoptosis is the blocking of the mitochondrial electron transport pathway and the change of the mitochondrial transmembrane potential. Therefore, in the next step, we will study the apoptosis mechanism of drug-resistant cells from VB12-sericin-PBLG-PTX to provide a more complete anti-tumor mechanism of VB12-sericin-PBLG-PTX.

## CONCLUSION

In order to improve the chemotherapy sensitivity of drug-resistant gastric carcinoma cells, this study conducted a study on the anti-gastric carcinoma effect of vitamin B12-conjugated nanocomposites. Successfully synthesized a new sericin nanomedicine (VB12-sericin-PBLG-PTX) with gastric carcinoma targeting. The nano micelles can significantly reduce the activity of gastric carcinoma drug-resistant cells, increase the apoptosis rate of gastric carcinoma drug-resistant cells, and achieve a good anti-tumor effect.

## DATA AVAILABILITY

The data used to support the findings of this study are included within the article.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## ACKNOWLEDGMENTS

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## SUPPLEMENTARY MATERIAL

Including original data of Partical size, Polymer dispersity index, Zeta potential. The original data of In vitro dissolution curve of nanomicelles, the original activity data of PTX on BGC-823 cells, the original activity data of VB12-sericin-PBLG-PTX on BGC-823 cells, the original activity data of PTXon BGC-823/DR cells, and the original activity data of VB12-sericin-PBLG-PTX on BGC-823/DR cells.

## REFERENCES

1. D.E. Guggenheim, and M.A. Shah,, Gastric cancer epidemiology and risk factors. *Journal of Surgical Oncology,* vol.107,no.3, pp.230–236, 2013.
2. P. Karimi, F. Islami, S. Anandasabapathy, N.D. Freedman, F. and Kamangar, Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. *Cancer Epidemiology, Biomarkers and Prevention,* vol.*23*,no.5, pp.700–713, 2014.
3. T.L. Ang, and K.M. Fock, Clinical epidemiology of gastric cancer. *Singapore Medical Journal,* vol.*55*,no.12, pp.621–628,2014.
4. F.M. Johnston, and M. Beckman, Updates on Management of Gastric Cancer. *Current Oncology Reports,* vol.*21*,no.8, pp.67, 2019.
5. M. Venerito, A. Link, T. Rokkas, and P. Malfertheiner, Gastric cancer - clinical and epidemiological aspects. *Helicobacter,* pp.39–44,2016.
6. BA. Digklia, and A.D. Wagner, Advanced gastric cancer: Current treatment landscape and future perspectives. *World Journal of Gastroenterology,* vol.*22*,no.8, pp.2403–2414,2016.
7. E.C. Smyth, M. Nilsson, H.I. Grabsch, et al., Gastric cancer. *Lancet,* vol.*396*,no.10251, pp.635–648,2020.
8. Japanese Gastric Cancer Association., Japanese gastric cancer treatment guidelines 2014 (ver. 4). *Gastric Cancer,* vol.*20*,no.1, pp.1–19,2017.
9. P.R. Kamath, and D. Sunil, Nano-Chitosan Particles in Anticancer Drug Delivery: An Up-to-

Date Review. *Mini Reviews in Medicinal Chemistry,* vol.*17*,no.15, pp.1457–1487,2017.
10. R.I.Kunz, R.M. Brancalhão, L.F. Ribeiro , et al., Silkworm Sericin: Properties and Biomedical Applications. *Biomed Res Int.* vol.2016,pp.8175701,2016
11. W.Guo, L.Deng, J.Yu, et al. Sericin nanomicelles with enhanced cellular uptake and pH-triggered release of doxorubicin reverse cancer drug resistance. *Drug Deliv.* vol.25,no.1,pp.1103-1116,2018.
12. B,A. Weaver, How Taxol/paclitaxel kills cancer cells. *Mol Biol Cell.* vol.25,no.18,pp.2677-2681,2014.
13. H. Malekinejad, S. Ahsan, F. Delkhosh-Kasmaie, et al., Cardioprotective effect of royal jelly on paclitaxel-induced cardio-toxicity in rats. *Iran J Basic Med Sci.* vol.19,no.2,pp.221-227,2016.
14. X.Sun, N.Wang, L.Y.Yang, et al. Folic Acid and PEI Modified Mesoporous Silica for Targeted Delivery of Curcumin. *Pharmaceutics.* vol.11,no.9,pp.430,2019.
15. J.Wang, J. Tan, J. Luo, et al. Enhancement of scutellarin oral delivery efficacy by vitamin B12-modified amphiphilic chitosan derivatives to treat type II diabetes induced-retinopathy. *J Nanobiotechnology.* vol.15,no.1,pp.18,2017.
16. F. O'Leary, S. Samman, Vitamin B12 in health and disease. *Nutrients.* vol.2,no.3,pp.299-316, 2010 .
17. J.F.H. Arendt, H.T.Sørensen, L.J. Horsfall, et al., Elevated Vitamin B12 Levels and Cancer Risk in UK Primary Care: A THIN Database Cohort Study. *Cancer Epidemiol Biomarkers Prev.* vol.28,no.4,pp.814-821,2019.
18. B.R. Sah, R.Schibli, R. Waibel, et al. Tumor imaging in patients with advanced tumors using a new (99m) Tc-radiolabeled vitamin B12 derivative. *J Nucl Med.* vol.55,no.1,pp.43-9,2014.
19. F.De Vita, C.Borg, G.Farina, et al Ramucirumab and paclitaxel in patients with gastric cancer and prior trastuzumab: subgroup analysis from RAINBOW study. *Future Oncology,* vol.15,no.23, pp.2723–2731,2019.
20. H.Ando, H.Shimodaira, K.Fujitani, et al. Olaparib in combination with paclitaxel in patients with advanced gastric cancer who have progressed following first-line therapy (GOLD): a double-blind, randomised, placebo-controlled, phase 3 trial. *The Lancet Oncology,* vol.18,no.12, pp.1637–1651, 2017.
21. Gynecologic Oncology Group, M. Markman, J. Blessing, et al., Phase II trial of weekly paclitaxel (80 mg/m2) in platinum and paclitaxel-resistant ovarian and primary peritoneal cancers: a Gynecologic Oncology Group study. *Gynecologic Oncology,* vol.101,no.3, pp.436–440,2006.
22. Z. Zhai, P. Xu, J. Yao,et al., Erythrocyte-mimicking paclitaxel nanoparticles for improving biodistributions of hydrophobic drugs to enhance antitumor efficacy. *Drug Delivery,* vol.27,no.1, pp.387–399, 2020.
23. N. Resman, A.Oblak, T.L. Gioannini, et al., Tetraacylated lipid A and paclitaxel-selective activation of TLR4/MD-2 conferred through hydrophobic interactions. *Journal of Immunology,* vol.192,no.4, pp.1887–1895,2014.
24. N.I. Marupudi, J.E. Han, K.W. Li, et al., Paclitaxel: a review of adverse toxicities and novel delivery strategies. *Expert Opinion On Drug Safety,* vol.6,no.5, pp. 609–621, 2007.
25. Y.R.Wang, S.Y. Yang, G.X. Chen, et al., Barbaloin loaded polydopamine-polylactide-TPGS (PLA-TPGS) nanoparticles against gastric cancer as a targeted drug delivery system: Studies in vitro and in vivo. *Biochemical and Biophysical Research Communications,* vol.499,no.1, pp.8–16, 2018.
26. Y.L.Fan, B.Y. Fan, Q. Li, et al., Preparation of 5-fluorouracil-loaded nanoparticles and study of interaction with gastric cancer cells. *Asian Pacific Journal of Cancer Prevention,* vol.15,no.18, pp.7611–7615,2014.
27. Y. Gao, Y. Shi, L. Wang, et al., Advances in mathematical models of the active targeting of tumor cells by functional nanoparticles. *Comput Methods Programs Biomed,* vol.184, pp.105106(1) – (44),2020.
28. S.Jin, Z. Du, H. Guo, et al., Novel Targeted Anti-Tumor Nanoparticles Developed from Folic Acid-Modified 2-Deoxyglucose. *International Journal of Molecular Sciences,* vol.20,no.3, pp.697,2019.
29. F. Ahsan, T.M. Ansari, S. Usmani, et al., An Insight on Silk Protein Sericin: From Processing to Biomedical Application. *Drug Research,* vol.68, no.6, pp.317–327, 2018.
30. N.Salunkhe, N. Jadhav, H. More, et al., Sericin Inhibits Devitrification of Amorphous Drugs. *AAPS PharmSciTech,* vol.20,no.7, pp.285, 2019.
31. K.Y.Cho, J.Y. Moon,, Y.W. Lee,, et al., Preparation of self-assembled silk sericin nanoparticles. *International Journal of Biological Macromolecules,* vol.32,no.1-2, pp.36–42,2003.
32. M. Watanabe, and T. Suzuki, Cadmium-induced abnormality in strains of Euglena gracilis: morphological alteration and its prevention by zinc and cyanocobalamin. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology,* vol.130,no.1, pp.29–39,2001.
33. M.F.Francis, M. Cristea, and F.M. Winnik, Exploiting the vitamin B12 pathway to enhance oral drug delivery via polymeric micelles. *Biomacromolecules,* vol.6,no.5, pp.2462–2467,2005.
34. H.Shirzadeh, G.R. Clarke, H.P. McNeil, et al., An IL-3-induced splenic NC-1.1+ mast cell line mediates natural cytotoxicity independent of TNF-alpha. *Cellular Immunology,* vol.174,no.2, pp.147–154,1996.
35. E.Pasquier, S. Honore, B. Pourroy, et al., Antiangiogenic concentrations of paclitaxel induce an increase in microtubule dynamics in endothelial

cells but not in cancer cells. *Cancer Research,* vol.65,no.6, pp.2433–2440, 2005.

36. S. Chen, Z. Song, and R. Feng, Recent Development of Copolymeric Nano-Drug Delivery System for Paclitaxel. Anti-Cancer Agents in Medicinal Chemistry, vol.20,no.18, pp.2169-2189,2020.
37. P. Ma, and R.J. Mumper, Paclitaxel Nano-Delivery Systems: A Comprehensive Review. *Journal of Nanomedicine & Nanotechnology,* vol.4,no.2, pp.e1000164(1)- e1000164 (35), 2013.