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Application of calcium carbonate based nano drug delivery system in cancer therapy

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Abstract: Calcium carbonate has great advantages in the construction of nano drug delivery systems due to its low cost and high biocompatibility. In this review, focusing on the requirements in cancer therapy, the advantages of calcium carbonate nanoparticles, the preparation of carbonate nanoparticles, and calcium carbonate nanoparticle-based nano drug delivery systems for the delivery of different types of cancer drugs were reviewed. The challenges of this system and future research directions were also summarized.

Key words: calcium carbonate; nano drug delivery system; cancer therapy

碳酸钙纳米给药系统在肿瘤治疗中的应用

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摘要：碳酸钙具有成本低廉、毒副作用低等优点，在构建纳米给药系统中具有极大的优势。文章围绕肿瘤治疗的应用需求，分别从碳酸钙纳米粒的优势、碳酸钙纳米粒的制备和递送不同类型肿瘤药物的碳酸钙纳米给药系统等 3 个方面进行综述，总结了纳米给药系统面临的困境和未来的研究方向。

关键词：碳酸钙；纳米给药系统；肿瘤治疗

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Nano drug delivery system, a recently emerging method of administration, have been proven to significantly enhance drug accumulation and retention in tumor tissue while decreasing the side effects of drugs[1-3]. Nano drug delivery systems can also increase the solubility, serum stability, and bio-

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availability of drugs[4]. By using proper drug delivery carriers, the aims of tumor targeting and responsive drug release can also be achieved, which are beneficial for cancer therapy[5].

Among the various materials employed to construct nano drug delivery systems, calcium carbonate is widely applied for the delivery of many drugs, which is gaining more and more attention in the field of cancer therapy[6]. Calcium carbonate shows several advantages over other currently available counterparts. The advantages include:

High biocompatibility, biodegradability and stability: Some widely adopted nanoparticles such as quantum dot and silver nanoparticles usually contain heavy metal elements, which may increase the reactive oxygen species (ROS) levels within cells[7] or induce direct DNA damage[8]. At the same time, the degradation process of many nanoparticles is slow, which may cause potential side effects upon administration. However, as the main component of bones, calcium carbonate shows high biocompatibility and biodegradability with negligible side effects in the recipients[9]. Due to its rigid structure, calcium carbonate does not undergo rapid degradation or structural failure upon transportation or dilution in physiological environments (usually non-acidic conditions) and usually show high stability upon delivery.

Ease of synthesis and surface modification with low cost: Calcium carbonate can be easily synthesized by co-precipitation methods using soluble calcium salts with carbonas. In some methods, the synthesis is free of organic solvents and can significantly decreases the cost[10-12]. In this way, drugs can be easily incorporated into the matrix of calcium carbonate with varying degrees of content-loading for different drugs (e. g., 1. 29% for p53 DNA and 0. 63% fordoxorubicin hydrochloride)[13]. The size of calcium carbonate can be freely tuned using different methods or by adjusting the reaction parameters. In this way, the size of the obtained calcium carbonate particles ranged from the nanoscale to the microscale[14-18]. The overexpression of Ca^{2+} on the surface of calcium carbonate makes it suitable for reacting with acid groups such as phosphate and carboxylate groups to realize surface modification[19-20].

pH-responsive degradation and drug release: As confirmed by many previous studies, calcium carbonate can degrade under acidic environments to release the loaded drugs. Since tumor tissues are usually mildly acidic, drug delivery using calcium carbonate is considered a promising way to realize cancer-specific drug release[21-22]. It was reported that calcium carbonate-mineralized polymer nanoparticles only released 34. 7% of the drug at pH 7. 4 in 24 h, while the ratio was significantly elevated to 57. 7% in an acetic acid buffer solution at pH 5. 0[23].

Therefore, calcium carbonate is a promising carrier for the construction of nano drug delivery systems. In this review, we aimed to summarize the application of calcium carbonate-based nano drug delivery systems in cancer therapy.

1　Synthesis of calcium carbonate

There are many ways to synthesize nanoscale calcium carbonate particles using microemulsion, co-precipitation, and vapor diffusion methods, the three mainly adopted methods.

Among the microemulsion methods, the reverse microemulsion method and the double emulsion method are the two most widely adopted approaches. They utilize water as a template in an oil reverse-microemulsion

and the reaction proceeds within the nanoscale water pools of the emulsion. Thus, the size of the as-prepared calcium carbonate can be easily regulated by adjusting the size of the reverse microemulsion[9].

In the co-precipitation method, calcium carbonate can be synthesized directly by the reaction between soluble calcium salts and carbonas. The size of calcium carbonate is affected by the stirring speed, solution pH, additives, and reactant concentrations and proportions. In the work performed by UENO et al. [24], calcium carbonate with a size of 100 nm could be obtained in an aqueous solution using high-speed stirring. WEI et al. [25] reported the synthesis of 500 nm sized calcium carbonate particles using starch as an additive under a relatively low reactant concentration.

In our previous work, we found that the vapor diffusion method was a convenient way tosynthesize different-sized calcium carbonate particles (Fig.1). In brief, calcium chloride was dissolved in ethanol and placed in a sealed container with ammonium carbonate or ammonium bicarbonate. The degradation of ammonium carbonate/ammonium bicarbonate generated ammonia and carbon dioxide, which diffused into ethanol to generate NH_4^+ and CO_3^{2-}. In the alkaline condition created by NH_4^+, Ca^{2+} and CO_3^{2-} in the ethanol generated amorphous calcium carbonate with good dispersity and narrow distribution. The size of calcium carbonate particles can be tuned by adjusting the charged weight of calcium chloride, the reaction time and temperature, and the water content of the system[26].

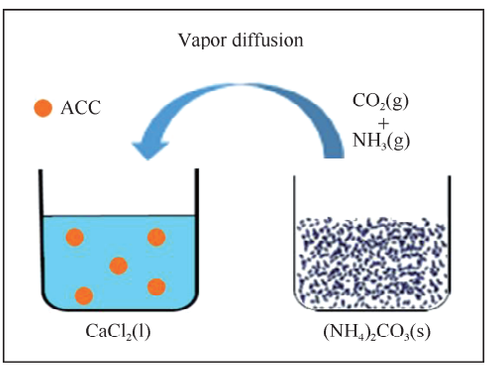

图 1 制备碳酸钙颗粒的气相扩散法

Fig.1 Vapor diffusion method to prepare calcium carbonate particles

2 Calcium carbonate-based nano drug delivery systems for anti-cancer drugs

Calcium carbonate-based nano drug delivery systems can be divided into three types based on the diversity of anticancer drugs.

2.1 Pure calcium carbonate-based nano drug delivery systems

Employing the acidic degradation profile of calcium carbonate, calcium carbonate is expected to regulate tumor pH to finally create a hostile microenvironment to inhibit the growth of tumors. SOM et al. [27] synthesized calcium carbonate particles with a size around 100 nm and injected them intravenously into HT108 tumor-bearing nude mice. After scheduled administration of calcium carbonate nanoparticles, the pH of the tumor microenvironment significantly increased with decreased tumor growth.

2.2 Delivery of small molecules

In our previous work, the anti-tumor drug doxorubicin hydrochloride was loaded into calcium carbonate particles using the vapor diffusion method. Then, the as-prepared drug-loaded nanoparticles were subjected to surface modification by oleic acid and polyethylene glycol to obtain a well-dispersed nano drug delivery system (Fig.2). This platform could quickly release the loaded cargo upon cellular

uptake by MCF-7 cells, which achieved anti-tumor effects comparable to those of free drugs[20].

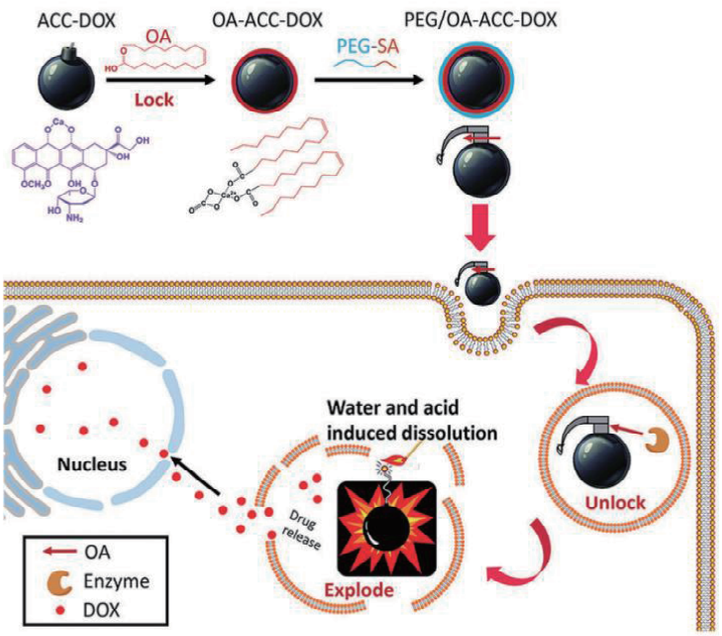

图 2　负载盐酸阿霉素的碳酸钙纳米粒的制备及作用机理[20]

Fig.2　Preparation of action mechanism of doxorubicin hydrochloride loaded calcium carbonate particles[20]

In another work, we also employed calcium carbonate for the dual-loading of the photosensitizer indocyanine green and doxorubicin hydrochloride. The dual-loaded nanoparticles were then surface-modified with phospholipid to obtain a nano drug delivery system with the proper size (100 nm). The platform could respond to near-infrared laser irradiation to induce the quick elevation of local temperature to accomplish the effective killing of cancer cells. By combining with the chemotherapeutic effect of doxorubicin hydrochloride, this platform resulted in better tumor inhibition performance than single therapies[28].

Recently, the calcium carbonate was also employed to load curcumin as a novel Ca^{2+} regulation system in the treatment of cancer (Fig.1). In this study, calcium carbonate was demonstrated to rapidly create Ca^{2+} within the acidic cancer cells while curcumin was utilized as a Ca^{2+} excretion inhibitor. Therefore, intracellular Ca^{2+} overload was triggered by this combination strategy and showed promising inhibition of the growth of cancer cells both in vitro and in vivo[29].

FAN et al.[30] prepared methylene blue (MB) loaded calcium carbonate using chemical precipitation method. In vitro experiments revealed that the loaded MB can generate $^1O_2$ under the irradiation of 650 nm laser. Moreover, due to the degradation of calcium carbonate, the produced CO_2 can afford in vivo ultrasonic imaging of tumor tissue for imaging guided drug delivery. Therefore, it provides a potential theranostics system with high biocompatibility for cancer therapy.

FENG et al.[31] synthesized mesoporous calcium carbonate nanoparticles, loaded with hemoporphyrin monomethyl ether (HMME) and then surface functionalized with hyaluronic acid. The platform was able to maintain stable under physiological environments and positively targeting to the tumor tissue due to hyaluronic acid mediated endocytosis. Most importantly, HMME can response to the activation of ultrasound and transfer the energy to the surrounding O_2 to create $^1O_2$, which realized

sonodynamic therapy of cancers.

2.3 Delivery of macromolecules

UENO et al.[24] employed a co-precipitation method to directly incorporate granulocyte-colony stimulating factor (G-CSF) into calcium carbonate nanoparticles. The as-prepared nano drug delivery system was able to achieve the sustained release of G-CSF for seven days and significantly increased the stability of G-CSF compared to free G-CSF. This represents a promising way for the encapsulation and delivery of active proteins for cancer therapy.

KONG et al.[32] prepared a multifunctional nanoparticle composed of calcium carbonate coated gold nanoparticles, acetyl dextran and palmitoyl oleoyl phosphatidyl choline, which can afford the loading of various proteins including amylase and HER-2 antibody. It was reported that the encapsulation efficiency of amylase was (82±3.5)% without compromising the activity of the enzyme. Moreover, the HER-2 antibody loaded system can exert positive inhibition effect on HER-2 positive SKBR3 cells, indicating the successful loading and release of proteins. This study reveals the potential of calcium carbonate as a versatile drug delivery carrier.

CHEN et al.[33] used facile chemical precipitation method to synthesize calcium carbonate with size around 900 nm under the regulation of arginine (termed as ACa nanoparticles). The calcium carbonate particle was then surface modified with polyethyleneimine (PEI) and employed as vehicle for the delivery of GFP labelled P53 DNA. The resulted system showed positive transfection on various cell lines, indicating the potential of calcium carbonate as vehicle for the construction of gene delivery systems (Fig.3). These results indicate that calcium carbonate can be employed as a suitable gene delivery carrier.

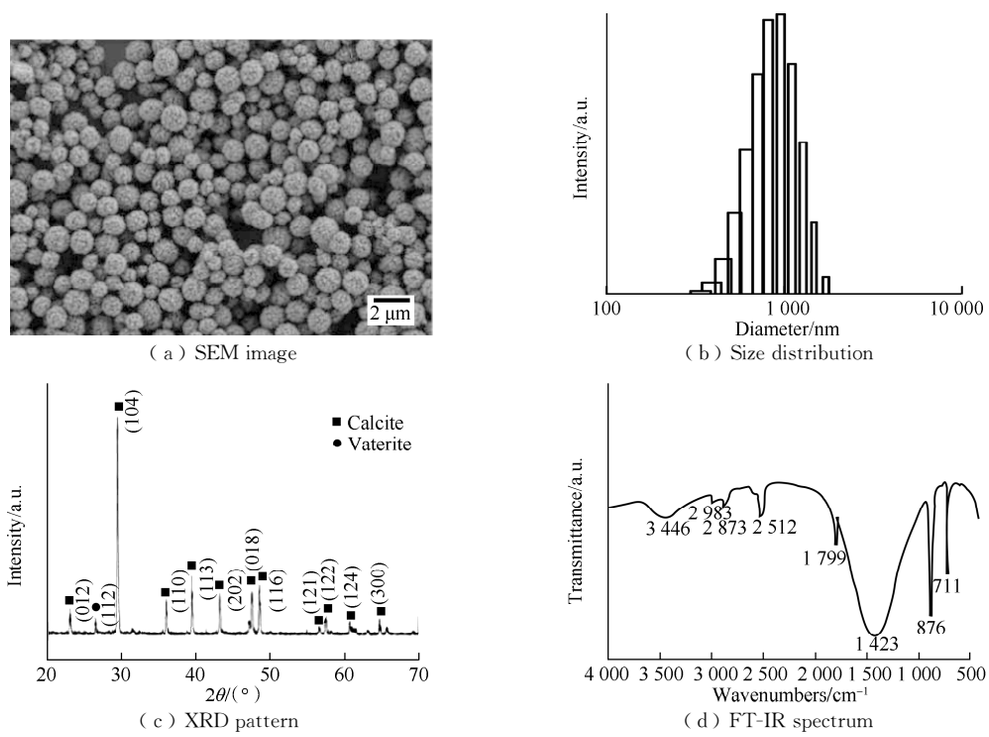

图 3 ACa 纳米颗粒的 SEM 图、粒程分布图、XRD 图谱和 FT-IR 光谱图

Fig.3 SEM image, size distribution, XRD pattern and FT-IR spectrum of ACa nanoparticles[33]

CHEN et al.[13] firstly prepared a mixed solution of doxorubicin hydrochloride, p53 DNA, and soluble calcium salt, and then employed a co-precipitation method to prepare doxorubicin hydrochloride and p53 DNA dual-loaded calcium carbonate nanoparticles. At a charged ratio of 37.5 (Ca^{2+} to CO_3^{2-}, molar ratio), the size of the obtained nanoparticles was around 100 nm. Moreover, in an experiment using HeLa cells, the platform showed promising gene transfection capacity and drug-delivery efficacy, with acceptable synergistic inhibition effects in HeLa cells.

In another work performed by ZHAO et al.[34], a reverse microemulsion method was adopted to prepare doxorubicin hydrochloride and miRNA-375 dual-loaded calcium carbonate nanoparticles. The nanoparticles were further subjected to the surface modification of lipids to construct a core-shell structured nano drug delivery system. Experiments with HepG2/ADR cells showed that the platform could effectively reverse the multidrug resistance of the cells and enhanced the intracellular accumulation of doxorubicin hydrochloride. Further in vitro and in vivo experiments also concluded that the combination of both drugs exhibited better anti-tumor performance than single therapies, which might decrease the administration dosage while preserving the curative effect to reduce the potential side effects.

3 Conclusion and perspective

Calcium carbonate nanoparticles have been widely used in the construction of nano drug delivery systems related to cancer therapy due to their characteristics of low cost, high availability, good biocompatibility, high stability, and responsive drug release. By means of the microemulsion, co-precipitation, and vapor diffusion methods, calcium carbonate particles with sizes ranging from nanometers to microns can be prepared[6,9] and used to load a series of anti-tumor drugs including doxorubicin hydrochloride, proteins, and nucleic acids. However, calcium carbonate nanoparticles also have some disadvantages, such as low drug-loading and easy aggregation, which might compromise the delivery efficacy of related drug delivery systems. In addition, the mechanisms of uptake, transport, degradation, and excretion of calcium carbonate-based nano drug delivery systems still remain largely unknown[35].

In future studies, in addition to the deficiencies mentioned above, the following issues need to be clarified for the further development and utilization of calcium carbonate-based nano drug delivery systems:

1) Explore the mechanisms of uptake, transport, degradation, and excretion of calcium carbonate nanoparticles and calcium carbonate-based nano drug delivery systems at both in vitro and in vivo. This is of vital importance for future development of related systems for cancer therapy.

2) Explore large-scale and controllable methods for industrial production and further reduce the cost of calcium carbonate, which is an important step toward the final marketing of calcium carbonate-based nano drug delivery systems.

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