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Review

Calcium Carbonate-Based Nanoplatforms for Cancer Therapeutics: Current State of Art and Future Breakthroughs

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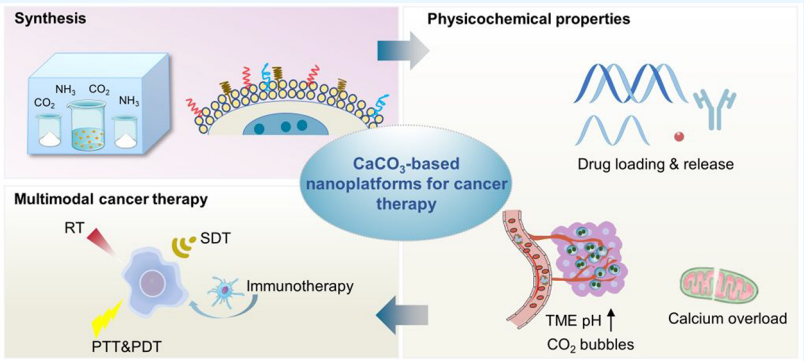

ABSTRACT: With the rapid development of nanotechnology, nanomaterials have shown immense potential for antitumor applications. Nanosized calcium carbonate ($CaCO_3$) materials exhibit excellent biocompatibility and degradability, and have been utilized to develop platform technologies for cancer therapy. These materials can be engineered to carry anticancer drugs and functional groups that specifically target cancer cells and tissues, thereby enhancing therapeutic efficacy. Additionally, their physicochemical properties can be tailored to enable stimuli-responsive therapy and precision drug delivery. This Review consolidates recent literatures focusing on the synthesis, physicochemical properties, and multimodal antitumor therapies of $CaCO_3$-based nanoplatforms (CBN). We also explore the current challenges and potential breakthroughs in the development of CBN for antitumor applications, providing a valuable reference for researchers in the field.

1. INTRODUCTION

Cancer is a prevalent disease threatening human health, and its treatment involves a variety of treatment modalities. With the continuous development of nanotechnology, nanoparticles (NPs) are increasingly being employed in cancer treatment, providing innovative strategies for drug transport, improving treatment efficiency, and alleviating side effects.[1,2] Recently, the application of inorganic nanoparticles in drug delivery has become increasingly prominent.[3,4] Nanoparticle drug delivery systems are particles between 1−500 nm in size that can deliver therapeutic drugs to specific targets in the body, thereby improving efficacy and reducing side effects. Among a variety of inorganic biominerals, due to their good biocompatibility and their superiority as an effective transport system for various types of drugs, calcium-based nanomaterials offer an exceptional range of advantages.[5,6] With the advancement of nanotechnology, Ca^{2+} overload-induced apoptosis can be modulated by functional nanomaterials, leading to the development of a new cancer therapeutic strategy (termed Ca^{2+} interference therapy).[7] Ca^{2+} overload-regulated tumor therapy does not demand any external stimuli such as light, heat and ultrasound, which is favorable for a widespread biological applications and clinical translation.[8,9] Hence, there has been a growing focus on tumor therapy mediated by Ca^{2+} overload. In addition, calcium-based nanomaterials are generally inexpensive due to their ease of production and low-cost raw materials, and have relatively high biosafety due to biocompatible components.[10]

Five prevalent types of nanomaterials based on calcium include: calcium phosphate (CaP), calcium carbonate ($CaCO_3$), calcium peroxide (CaO_2), calcium hydride (CaH_2), and calcium sulfide (CaS).[9] Of these materials, calcium carbonate ($CaCO_3$) is the most commonly utilized in calcium-based nanotechnology.[11] Recently, $CaCO_3$-based nanoplatforms (CBN) as drug delivery systems (DDSs) in cancer treatment have received considerable attention because

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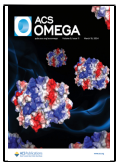

Scheme 1. Schematic Illustration of the Synthesis, Physicochemical Properties and Multimodal Antitumor Therapies of $CaCO_3$-Based Nanoplatforms (CBN)

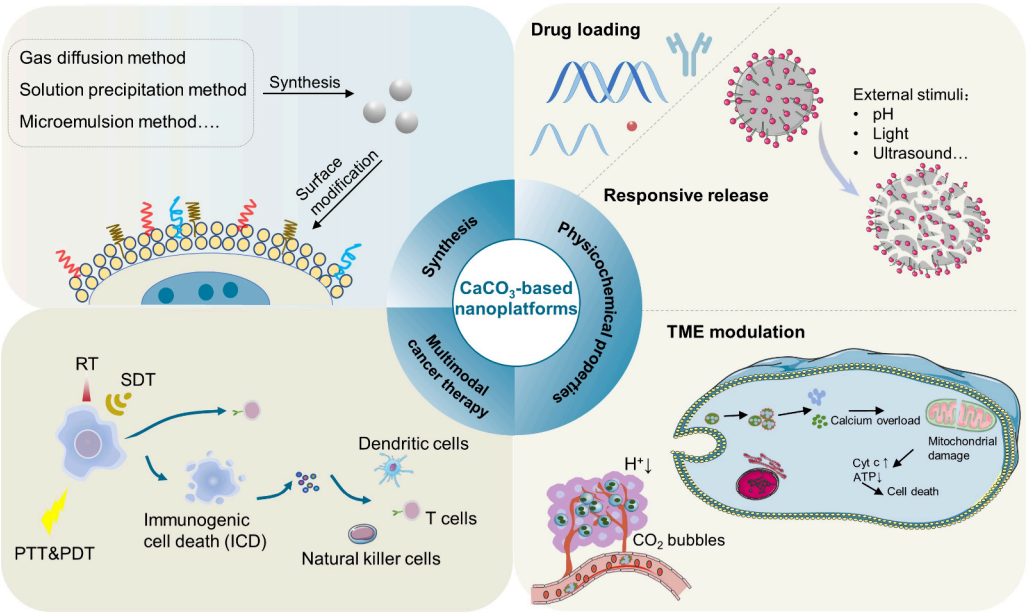

Table 1. Comparison of the Main Methods of $CaCO_3$-Based Nanoplatforms Synthesis

Typical methods	Principle	Pros and cons	ref
Precipitation method	Calcium and carbonate ions react in water, precipitating as solid $CaCO_3$	Pros: Easy, affordable, scalable, and adjustable particle size Cons: Potential particle inconsistency, limited crystal control	10,19
Gas diffusion method	Gaseous CO_2 reacts with dissolved Ca^{2+} in water to precipitate $CaCO_3$ NPs	Pros: Precise particle shape control, high purity of NPs Cons: Complex setup, slower production	26,30
Flame synthesis method	Calcium-rich precursors combust to form $CaCO_3$ NPs in the Flame Synthesis Method	Pros: Fast NPs production, good for mass production Cons: Safety risks at high temperatures, variable particle sizes	31
Cockle shell decomposition Method	$CaCO_3$ NPs are obtained from Cockle shells mainly by mechanical grinding	Pros: Natural source use, eco-friendly, organic incorporation Cons: Organic residue may affect purity, potentially slower process	32
Biomineralization method	Biological entities guide $CaCO_3$ NPs crystallization in calcium-rich solutions	Pros: Green method, structured crystals, high purity and biocompatibility Cons: Slow reaction rate, precise biological control requirement	33

of their low cost, biosafety, biodegradability, and pH sensitivity.[12−14] These DDSs enable the sustained and direct release of the targeted therapeutic agent. In particular, for protein and gene delivery and ultrasound (US) image-guided therapy, CBN have great potential.[12,15] Another aspect of their potential use is the functionalization of CBN, which is mainly related to providing active targeting, increasing stability, and improving the ability to load the drug. In addition, the robust buffer function of CBN makes them especially competitive in alleviating the acidic tumor microenvironment (TME), which is one of the factors that most CBN may be involved in modulating tumor immunity.[16] Most importantly, since the degradation products consist only of calcium (controlled by kidneys and accumulated in bones)[17] and carbon dioxide (CO_2, excreted by the lungs),[18] the side effects of using CBN to treat tumors can be negligible.

In this Review, we summarize the development of CBN for cancer therapy. We focus on the synthesis, physicochemical properties (drug loading capabilities, responsive release, and their effects on the TME) of CBN and $CaCO_3$-based multimodal antitumor therapies (Scheme 1). We also discuss the current problems and possible future breakthroughs in the use of CBN in antitumor applications. This Review can help researchers quickly understand the antitumor mechanism and research status of CBN, and it points out the direction for their future research.

2. SYNTHESIS OF $CACO_3$-BASED NANOPLATFORMS

2.1. Typical Methods to Prepare $CaCO_3$ NPs. At present, common approaches for the preparation of $CaCO_3$ NPs mainly include precipitation method, gas diffusion method, flame synthesis method, cockle shell decomposition method, biomineralization method, etc. (Table 1).[10,11,19] Among them, $CaCO_3$-based drug delivery systems typically use the solution precipitation method, microemulsion method and gas diffusion method (Figure 1).[14,20,21] We will describe these synthesis methods in detail below.

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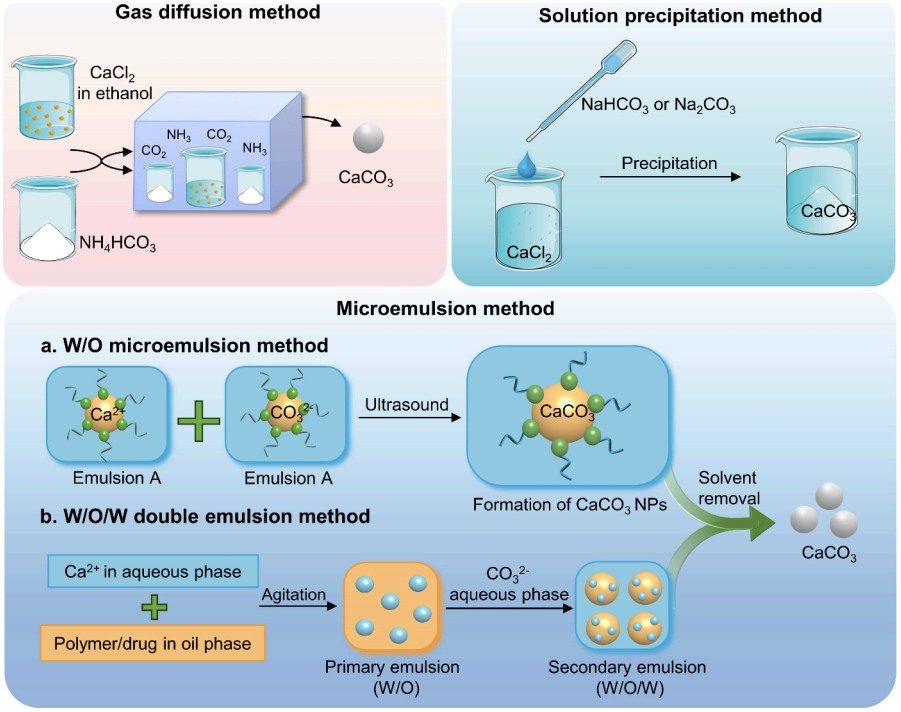

Figure 1. Synthesis of $CaCO_3$ NPs. Schematic illustration of the typical methods for preparing $CaCO_3$ NPs used in cancer therapy: gas diffusion method, solution precipitation method, and microemulsion method.

The most well-known method for the production of $CaCO_3$ NPs is the solution precipitation method, utilizing the reaction between Ca^{2+} and CO_3^{2-} in aqueous solution. This method enables the mass production of $CaCO_3$ NPs without the use of surfactants, thereby reducing the cost of production. Due to the mild conditions of preparation, many biologically active substances can be loaded into $CaCO_3$ NPs in the process of precipitation, including small molecule drugs, genes, and proteins. For example, Vidallon et al. utilized rapid precipitation technology combined with biocompatible polymer additives such as bovine serum albumin (BSA) and polydopamine (PDA) to create $CaCO_3$ hybrid particles.[22] By using BSA and PDA, hybrid particles could have different sizes and polymorphic compositions through different reaction or mixing times. The physicochemical properties of the resulting $CaCO_3$ hybrid particles could be adjusted by controlling other manufacturing process parameters, including PDA polymerization time, salt addition sequence, and salt-polymer additive pairing. These findings are important for the development of hybrid particle systems with customized properties for specific applications such as contrast-enhanced US and photoacoustic imaging, drug transport, and cancer treatment.

As an extension of the precipitation method, the microemulsion method is widely used in the preparation of $CaCO_3$ NPs and gene encapsulation. The microemulsion method includes water-in-oil (W/O) reverse microemulsion method and double microemulsion method.[12,23] The reverse microemulsion uses W/O microemulsion droplets as a nanoreactor.[24] First, "calcium microemulsion" and "carbonate microemulsion" are prepared by adding a Ca^{2+} or CO_3^{2-} aqueous phase to the organic phase, respectively. Then, the "calcium microemulsion" is mixed with the "carbonate microemulsion" to form $CaCO_3$ NPs. Finally, the $CaCO_3$ NPs are separated by a centrifuge. Khan et al. prepared pH-sensitive lipid coated cisplatin (CDDP)/oleanolic acid (OA) $CaCO_3$ NPs (CDDP/OA-LCC NPs) using a microemulsion method.[21] In vitro release experimental results showed that more drugs were released from CDDP/OA-LCC NPs under acidic pH than under alkaline pH, making them an ideal carrier for pH-sensitive chemotherapeutic drugs. In the double microemulsion method, a W/O "calcium microemulsion" is obtained by the inverse microemulsion method, and then a large amount of water phase composed of CO_3^{2-} is mixed with the "calcium microemulsion", resulting in the formation of a W/O/W double emulsion. In the W/O/W double emulsion, $CaCO_3$ NPs are formed through the reaction of Ca^{2+} and CO_3^{2-}.[12]

Amorphous calcium carbonate (ACC) filled with small drug molecules is mainly produced by the gas diffusion method.[25] As shown in Figure 1, $CaCl_2$ is dissolved in ethanol, and the solution is transferred to a glass vial. Then, two bottles of $CaCl_2$ and ammonium bicarbonate are placed in the desiccator. The ammonium bicarbonate can produce CO_2 and NH_3. Then CO_2 and NH_3 can form CO_3^{2-} and NH_4^+ when they dissolve in an ethanol solution. Under an alkaline NH_4^+ environment, CO_3^{2-} reacts with Ca^{2+} to generate ACC. Compared with other synthesis methods producing $CaCO_3$ with good crystallization, the advantage of the gas diffusion method is that the obtained $CaCO_3$ NPs can load more pharmaceuticals.[26] It is worth mentioning that the size, shape, and phase of $CaCO_3$ NPs produced by these methods can be tuned by changing synthesis factors such as pH, reaction temperature, ionic concentrations and surfactants.[27−29]

2.2. Surface Modification of $CaCO_3$ NPs for Cancer Therapy. Like other biomedical nanomaterials, proper surface modification can make $CaCO_3$ nanomaterials play a better role under physiological conditions, such as preventing agglomeration, increasing stability, targeting, biocompatibility, and so on.[34] In the antitumor research, many materials are used to modify the surface of $CaCO_3$ nanomaterials, such as

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polyethylene glycol (PEG), liposomes, folic acid (FA), lipid, hyaluronic acid (HA), polyethylenimine (PEI) and so on.[35−38] Macromolecules such as nuclei acids including siRNA,[17,39] cancer targeting agents which include peptides and antibodies,[40,41] and polymers for example PEG, PEI and HA, have been used in cancer therapy. Recently, Wu et al. have constructed G/A@$CaCO_3$ NPs composed of glucose oxidase (GOX) and 2D antimonene quantum dots (AQDs) encapsulated in $CaCO_3$, which were coated using a constitutive lipid bilayer and additionally PEG to enhance physiological stability.[37] In another study, Wang et al. reported that mesoporous silica NPs (MSNs) modified with $CaCO_3$ and a lipid bilayer membrane (MSNs@$CaCO_3$@liposomes) could enable continuous drug release on TME and enhance biocompatibility. The $CaCO_3$ membrane played a guiding role by closing the MSN pore to allow pH-triggered release of the drug to enter into the cancer cell. In addition, a lipid bilayer encapsulated by MSNs@$CaCO_3$ enhanced cellular uptake and biocompatibility.

3. PHYSICOCHEMICAL PROPERTIES OF $CACO_3$-BASED NANOPLATFORMS

3.1. Diverse Drug Loading Capabilities. The therapeutic effect of antitumor drugs usually relies on high-dose and high-frequency administration, which can lead to serious side effects and toxicity for the organism.[42,43] To address this problem, drug carriers that deliver adequate amounts of drug to the site of action and maintain therapeutic levels over the course of treatment are often used.[44,45] In addition, drug encapsulation strategies have the potential to improve the delivery of compounds with poorly soluble, poorly stable, and high cytotoxicity under physiological conditions. Nanocarriers have several potential advantages in drug delivery:[46,47] (i) biocompatibility without significant toxicity or immune response; (ii) biodegradability in biological environments; (iii) responsiveness to low pH; (iv) easily available at low cost; and (v) stable biochemical properties that do not affect the biological activity of the payload. Due to cost-effectiveness, biosafety, biodegradability, and pH sensitivity,[48,49] $CaCO_3$ NPs can carry a variety of drugs that have killing effects on tumors, such as targeted drugs, nontargeted drugs, and genes.[50,51] They have good drug loading capacity and can maintain good drug stability.

3.1.1. Loading Targeted Drugs/Genes. $CaCO_3$ NPs can load both hydrophobic and hydrophilic molecules (such as chemotherapy drugs, nucleic acids and other functional molecules), making them suitable carriers for chemotherapy, gene therapy, photodynamic therapy (PDT), etc.[15] For example, Feng et al. developed a simple, rapid, and controllable industrial method to synthesize high-drug-loaded vaterite hollow calcium carbonate (VHC).[52] In the industrial preparation of $CaCO_3$, VHC can be prepared by using L-leucine. The different reaction conditions and the possible mechanism of VHC formation were studied in detail. The VHC exhibited good biocompatibility and had a high drug loading capacity (39.68%) for doxorubicin (DOX) due to the presence of hollow nanocavities. Meanwhile, DOX@VHC exhibited good pH responsiveness for continuous release of DOX. The cytostatic rate of DOX@VHC was 91.73%, indicating that this drug could significantly inhibit tumor cells and reduce damage to normal cells. The drug-loaded VHC could provide controlled drug release and effective inhibition of tumor cells, so it has good prospects for use in cancer therapy. Gene therapy also often plays a role in tumor treatment by replacing or silencing defective genes. However, nucleic acid delivery is very difficult due to their negatively charged, large size, and prone to degradation.[53] In a recent study, $CaCO_3$ NPs could be combined with nucleic acids, which can provide a promising vector for gene therapy. Through activation of the cyclic GMP-AMP synthase-stimulator (cGAS-STING) pathway of interferon genes, cytoplasmic double-stranded DNA (dsDNA) could elicit anticancer immunity.[54] In another study, Li et al. reported biomineralized growth of $CaCO_3$ NPs to synthesize cGAS-STING agonists. The obtained DNA@$CaCO_3$ could activate the cGAS-sting pathway in dendritic cells, making it a powerful immunostimulant.[55] After intratumoral injection, DNA@$CaCO_3$ effectively suppressed CT26 and B16-F10 tumors in mice by stimulating innate and acquired immunity to reverse the immunosuppressive TME. In addition, through biomineralization of complex $CaCO_3$-based tumor lysates, an individualized autologous tumor vaccine with cGAS-STING activation was created, which could elicit tumor-specific immunity that not only slows down tumor growth but also synergistically suppress postoperative tumor recurrence after surgery with anti-PD-1 immunotherapy. These results demonstrate a $CaCO_3$-based biomineralization approach for the preparation of autologous tumor vaccines in a short period of time, which has the potential for individualized immunotherapy and clinical applications.

Aparting from carrying drugs that cause direct damage to tumor cells, $CaCO_3$ NPs can also carry drug molecules that are responsive to the external environment, thus enabling spatiotemporally selective treatment of tumors.[56] For example, in order to combat castration-resistant prostate cancer (CRPC), Tan et al. constructed a TME-triggered nanoprobe (PGP/$CaCO_3$@IR820/DTX-HA).[35] The outer layer of $CaCO_3$ effectively encapsulated the chemotherapeutic agent docetaxel (DTX) and photosensitizer IR820 on the surface of pentagonal gold prisms (PGPs), acting as a TME activator to release TME-responsive drugs. The loaded photosensitizer IR820 could exert its killing effect on tumors when activated by external (Near-Infrared Radiation) NIR-light irradiation. After hyaluronic acid modification, PGP/$CaCO_3$@IR820/DTX-HA could synergize TME-activated photothermal therapy (PTT)/PDT/CT and tumor targeting transport. Therefore, PGP/$CaCO_3$ @IR820/DTX-HA has great potential for clinical translation by acting as a treatment for CRPC.

3.1.2. Loading Ultrasmall NPs. In addition to carrying molecules and protein drugs, $CaCO_3$ NPs can also load various ultrasmall NPs to achieve combination therapy. In some cases, the $CaCO_3$ component in the nanocomposites is used as a coating material to prevent the leakage of nanodrugs and protect the activity of the material.[35] Several studies have explored $CaCO_3$ nanocomposites loaded with NPs for cancer therapy, including Fe_3O_4@$CaCO_3$, $CaCO_3$−$MnSiO_x$, Cu_2O@$CaCO_3$, and others.[57−59] For example, a novel nanocatalyst consisting of $CaCO_3$-loaded GOX and two-dimensional antimony quantum dots (AQDs) was constructed and coated by a lipid bilayer (G/A@$CaCO_3$−PEG).[37] This nanocatalyst could prolong blood circulation and remain stable under physiologically neutral conditions but decompose under a weakly acidic TME, leading to rapid drug release in the TME. After integration, GOX could effectively catalyze glucose consumption, reducing adenosine triphosphate (ATP) production and subsequently down-regulating the expression of

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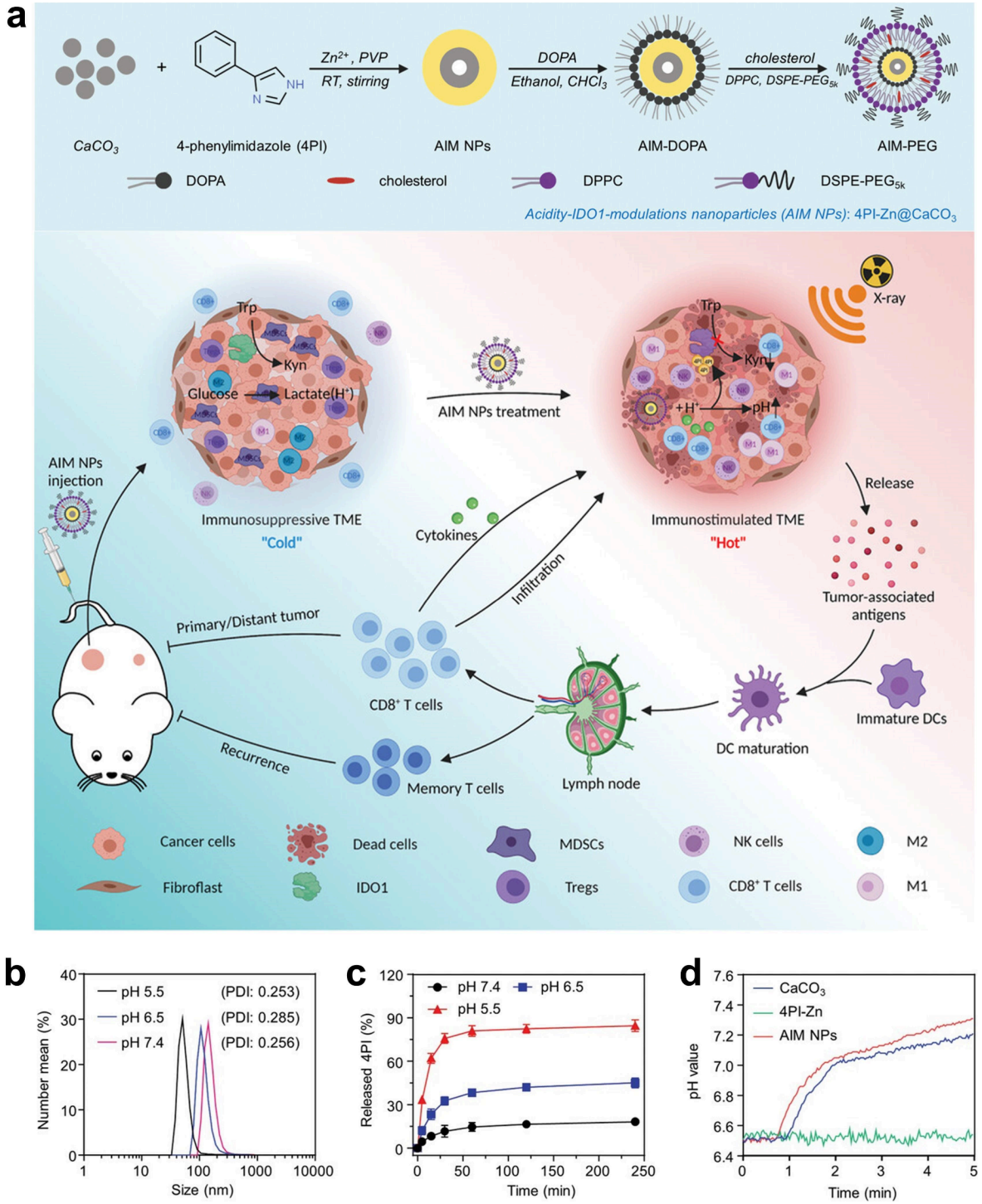

Figure 2. Acid neutralization by CBN. (a) Scheme illustrating the utilization of AIM NPs to reverse the immunosuppressive metabolic TME for reinforced radiation therapy. Upon tumor accumulation, these intravenously injected AIM NPs can react with protons to neutralize tumor acidity and instantly release the 4PI molecule to inhibit IDO1-mediated tryptophan (Trp) metabolism. (b) Hydrodynamic size distribution profiles of AIM NPs incubated in buffer solutions at different pH values. (c) Time-dependent release profiles of 4PI from AIM NPs incubated in buffer solutions at different pH values. (d) In vitro acidity neutralization profiles of $CaCO_3$, 4PI-Zn, and AIM NPs. Figure reproduced with permission from Wiley-VCH GmbH,[25] Copyright 2021.

heat shock proteins (HSPs). This effect, combined with the reversal of thermotolerance in cancer cells, enhanced the therapeutic effect of 2D AQDs-induced PTT under near-infrared (NIR) light activation. The results showed that G/A@$CaCO_3$−PEG had high antitumor activity, with an inhibition rate of 83.92%. This strategy provides a promising approach for enhancing hyperthermia-based cancer therapy by limiting the ATP supply.

3.2. Responsive Release Capability. $CaCO_3$ NPs can regulate pharmacokinetics by controlling drug release, thereby improving therapeutic efficacy and reducing side effects.[60] Usually, $CaCO_3$ NPs can release drugs through diffusion,

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carrier dissolution and recrystallization.[19] Due to the controlled release of various active substances loaded, $CaCO_3$-based nanocomposites can achieve precise cancer treatments by external stimuli, such as heat, light, and ultrasound, as well as internal stimuli, such as redox, pH, and enzymes.[61−64]

3.2.1. pH Responsiveness. The biological systems have different pH conditions, such as tumor tissues (pH 6.5−6.8) and normal tissues (pH 7.2−7.4).[65] pH is an important factor in the decomposition of $CaCO_3$ NPs. The free protons under acidic conditions can react with $CaCO_3$ NPs to produce CO_2, which can then dissolve the $CaCO_3$ NPs and accelerate drug release. The pH response of $CaCO_3$ NPs decomposes and releases drugs, which is the earliest and most studied.[16] Recently, Ding et al. constructed pH-sensitive $CaCO_3$ nanocarriers loaded with tumor cell lysates (TCL) and CpG immunostimulatory oligodeoxynucleotide 1826 for triple-negative breast cancer (TNBC) immunotherapy.[66] This specially designed nanovaccine $CaCO_3$@TCL/CpG could consume lactic acid to neutralize the acidic TME, increasing the M1/M2 ratio and promoting immune cells infiltration. In addition, this nanovaccine could activate tumor dendritic cells and recruit cytotoxic T cells to eliminate tumor cells. In vitro and in vivo experiments demonstrated that this nanovaccine had high cytotoxicity in 4T1 cells and significantly inhibited tumor development in mice. In another study, CRISPR/Cas9 gene-editing nanoplatforms that respond to dual stimulation of pH/glutathione could be synergized with Ca^{2+}-enhanced CO gas for precision cancer treatment.[64] In the TME, the rapid biodegradation of the $CaCO_3$ layer induced by pH could promote the production of H_2O_2 through the GOx-catalyzed oxidation of glucose, and then the generated H_2O_2 could further react with manganese carbonyl (MnCO) to release CO gas. At the same time, the Ca^{2+} overload caused by decomposition of $CaCO_3$ disrupted the intramitochondrial calcium balance, leading to Ca^{2+}-induced (reactive oxygen species) ROS production and activation of the mitochondrial pathway of apoptosis. Next, disulfide bonds induced by GSH were cleaved, and released Cas9/sgRNA (RNP) could ablate nuclear factor E2-related factor 2 (Nrf2) gene by interfering with ROS signaling to sensitize gas therapy. This therapeutic modality demonstrates the intelligent control function of CRISPR, ions, and gas codelivery, and provides novel ideas for precise treatment with nanomedicines.

3.2.2. Ultrasound (US)-Triggered Drug Release. US is one of the most powerful noninvasive imaging diagnostic tools available. Because of the similarity of US properties, it is difficult to distinguish between tumors and normal body tissue.[20] Therefore, $CaCO_3$ NPs have been used to construct contrast enhancement agents to improve the resolution of US imaging. In ultrasound imaging, the $CaCO_3$-based contrast agent showed strong echo signals and excellent echo persistence in tumor tissue.[67] In order to solve the low penetration efficiency of drugs in tumors, Chiang et al. constructed $CaCO_3$-DOX silica NPs by surface modification with fluorination.[68] A phospholipid layer (ADSFL NPs) was used to coat the surface of fluorinated $CaCO_3$-DOX silica NPs to attenuate aggregation and enhance the biocompatibility. Compared with the uncoated group, the outer shell of ADSFL NPs could generate interfacial nanobubbles induced by the superhydrophobic layer, which reduced the chance of DOX leakage into water by 7-fold and induced cavitation upon ultrasonic treatment. Furthermore, DOX release from ADSFL NPs could be triggered by the US treatment, which was further enhanced 1.6 times under acidic conditions. In vivo experimental results displayed that US could induce the cavitation activity of ADSFL NPs and cause vessel rupture, with a significant increase in the DOX fluorescence intensity at tumor sites.

3.2.3. Phototriggered Drug Release. Some CBN can also accelerate drug release under the stimulation of external light sources. A common strategy is that $CaCO_3$ nanocomposites are loaded with materials that can produce heat under light irradiation.[69] In a recent study, Wang et al. constructed $CaCO_3$-DOX@silica-indocyanine green nanospheres with a high degree of homogeneity and monodispersity, proposing a light-triggered strategy that could be used to treat drug-resistant tumors.[70] Under NIR laser irradiation, the nanospheres exhibited photothermal and photodynamic effects by converting laser energy to localized heat and ROS via linked indocyanine green molecules. Furthermore, light can induce drug release from nanospheres, giving the nanospheres chemotherapeutic properties. Eventually, this combination therapy realized the treatment of drug-resistant breast cancer cells. This potentially promising platform for improved multimodal cancer therapy could be provided by these $CaCO_3$-based phototriggered materials.

3.3. Effects on Tumor Microenvironment. Besides drug loading, CBN also plays several important roles in TME modulation, such as providing CO_2,[58] adjusting pH,[71] and serving as a Ca^{2+} supercarrier,[72] which induces immunogenic cell death (ICD) and autophagy thereby activating immunotherapy.

3.3.1. Acid Neutralization. $CaCO_3$, as a biocompatible biomineral, can react with protons in the TME, thereby neutralizing tumor acidity and then enhancing the efficacy of tumor therapy. For instance, Wang et al. designed pH-responsive nanomedicines prepared by coating $CaCO_3$ NPs with 4PI−Zn coordination organic polymers to reprogram the immunosuppressive metabolic TME, thereby enhancing the killing therapeutic effect of radiotherapy (Figure 2).[25] The obtained 4PI−Zn@$CaCO_3$ NPs (referred to as acidity-ido 1-modulated nanoparticles, AIM NPs) exhibited excellent pH-dependent dissociation and 4PI release behaviors, showing a role in reversing acidity-induced radiation resistance, respectively. In another study, Zhang et al. synthesized a novel embolic agent $CaCO_3$-coated alginate microspheres ($CaCO_3$-ALG MSs).[73] As $CaCO_3$ reacted with protons, this $CaCO_3$-ALG MSs could neutralize the pH of the tumor after $CaCO_3$ decomposition without affecting the whole morphology of the microspheres. Then transcatheter arterial embolization (TAE) treatment was performed using $CaCO_3$-ALG MSs in an orthotropic rat hepatocellular carcinoma model. 18F-fluorodeoxyglucose microposition emission tomography/CT imaging was performed after TAE and intra-arterial administration of $CaCO_3$-ALG MSs was found to significantly improve treatment efficacy. Further studies also confirmed that after treatment of TAE with $CaCO_3$-ALG MS, the reversal of from immunosuppression to nonimmunosuppression of TME occurred. This study not only proposes a novel calcium-based embolic agent for improved TAE therapy in hepatocellular carcinoma but also demonstrates a clinically relevant method to treat tumors through neutralizing tumor acidity. It is important to note that that when $CaCO_3$ nanocomposites are used to change the TME from weakly acidic to neutral, the efficacy of the drug or NPs carried by

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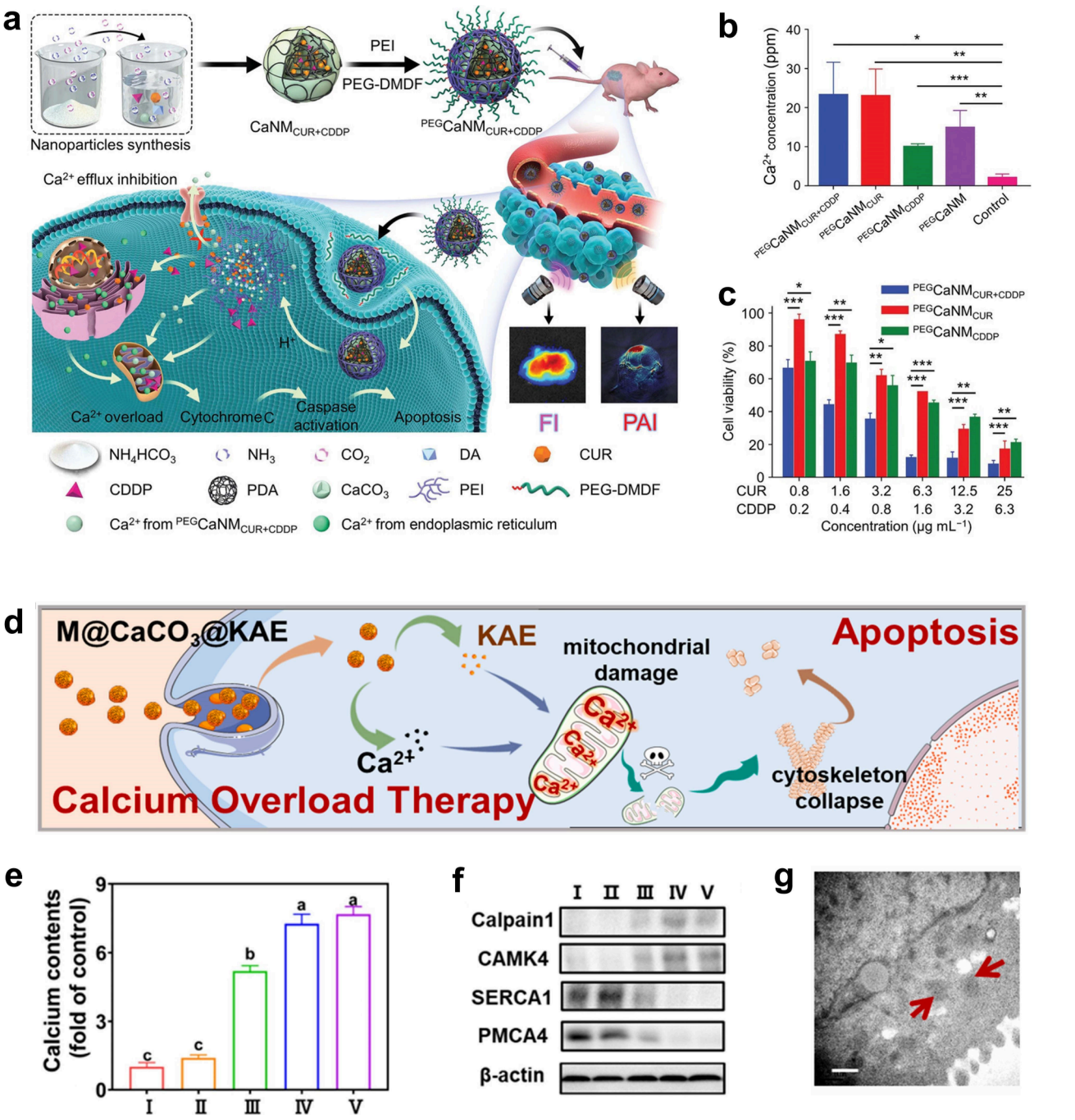

Figure 3. Calcium overload cancer therapy based on CBN. (a) Schematic representation of the functional pattern of multifunctional Ca^{2+} nanomodulator $^{PEG}CaNM_{CUR+CDDP}$. (b) Mitochondrial Ca^{2+} concentrations and (c) cell viabilities of MCF-7 cells after treatments with various Ca^{2+} nanomodulators. Figure reproduced with permission from Wiley-VCH GmbH,[72] Copyright 2021. (d) Schematic illustration of M@$CaCO_3$@KAE NP-mediated apoptosis. (e) The calcium contents of A549 cells. (f) Calcium regulated protein expression levels of A549 cells, I: Control; II: $CaCO_3$ NPs; III: KAE; IV: $CaCO_3$@KAE NPs; V: M@$CaCO_3$@KAE NPs. (g) TEM images of mitochondria from A549 cells treated with M@$CaCO_3$@KAE NPs. Figure reproduced with permission from Elsevier BV,[79] Copyright 2021.

these nanocomposites is expected to remain unaffected within this neutral setting.

3.3.2. Calcium Overload. Calcium ions (Ca^{2+}) play an important role in the regulation of many cellular functions. If intracellular Ca^{2+} levels exceed the amount tolerated by the cell (called Ca^{2+} overload), oxidative stress, mitochondrial damage and subsequently cell death can occur.[74] Thus, Ca^{2+} overload has been intensively developed as a novel cancer treatment strategy due to its high potency and desirable safety. CBN as Ca^{2+} overload regulators have acid-responsive properties, which means they can increase intracellular Ca^{2+} levels and induce cell apoptosis and death by releasing large amounts of Ca^{2+} in response to the TME.[7] However, due to the insufficient presence of Ca^{2+} at the tumor site, the entry of calcium into the tumor is inefficient, leading to limited therapeutic effects.[75] In order to fully utilize the Ca^{2+} in $CaCO_3$, bioactive substances such as curcumin (CUR) and capsaicin, exogenous physical stimuli such as ultrasound (US),

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and ROS can be employed to enhance Ca^{2+} overload.[76−78] For instance, Zheng et al. used the in situ PDA as a template in a sealing container to prepare a multichannel Ca^{2+} nanoregulator ($CaNM_{CUR+CDDP}$) conjugated $CaCO_3$ NPs combined with CUR to induce mitochondrial dysfunction by Ca^{2+} overload (Figure 3a-c).[72] After the whole body was administered, PEG modified $CaNM_{CUR+CDDP}$ efficiently accumulated in tumor sites, entered cancer cells, and induced the mitochondria damage, which significantly enhanced mitochondrial targeting tumor suppression through the outbreak of Ca^{2+} release, CUR-triggered Ca^{2+} efflux inhibition, and chemotherapy cisplatin (CDDP). The CUR of fluorescent imaging combined with the PDA of the light sound imaging was conducive to the visualization of nanomodulators. The simple and practical design of this multichannel Ca^{2+} nanoregulator will help the development of multimode biological imaging guidance in the future.

Furthermore, to enhance Ca^{2+} overload, promoting Ca^{2+} influx is also an effective strategy. Yamanol-3-O-Puchantoside (KAE) is a biologically safe flavonoid with excellent anticancer capabilities, capable of disrupting calcium homeostasis and effectively promoting internal calcium flow. Inspired by these concepts, Li et al. constructed KAE-loaded $CaCO_3$ NPs and combined with cell membrane (M) for synergistic tumor therapy (Figure 3d-g).[79] In this treatment platform (M@$CaCO_3$@KAE), the membrane coating ensured the targeted delivery of $CaCO_3$@KAE. Upon arrival at the tumor site, $CaCO_3$@KAE accumulated in the TME, releasing KAE and Ca^{2+}. KAE effectively disrupted the Ca^{2+} balance, while the levels of Ca^{2+} were significantly increased, and Ca^{2+} overload was magnified by the mediation of KAE. As a result, mitochondrial dysfunction occurred, leading to cell death in cancer cells. Through these combined effects, in vitro experiments demonstrated that M@$CaCO_3$@KAE achieved tumor suppression. This study presents an alternative nanoplatform acting as a "calcium bomb" to ensure the targeting and effectiveness of tumor therapy.

3.3.3. Generation of CO_2 Bubbles. CO_2 is generally regarded as a nontoxic, chemically inert substance that is highly soluble in blood and tissues, making it readily absorbed in cases of gas embolism.[80] Clinically, CO_2 has been widely used as an insufflation gas in laparoscopic surgery for diagnosis and treatment of intra-abdominal and gynecological diseases.[81,82] The extracellular pH of tumors is known to be lower than that of normal tissues, making them potential targets for therapy. Carbonic acid, exciting metal extraction, and absorption can be substituted and confirmed by a carbon regime. The generated CO_2 can promote drug release, aid in US imaging, rupture cell membranes, and act as a drug carrier, among other functions. The CO_2 produced by the decomposition of $CaCO_3$ can further promote the release of drugs within the $CaCO_3$. Leveraging this unique property, a $CaCO_3$-based nanosystem was proposed that can generate CO_2 gas and promote the release of encapsulated DOX at the tumor site.[67]

Due to the acoustic properties of CO_2 bubbles, they can serve as a good ultrasound contrast agent, providing a novel diagnostic method for tumor treatment.[83] For instance, to implement photoacoustic (PA), ultrasound, and thermal imaging guided PTT, Wan et al. prepared biomimetic pH-responsive nanoprobes using tumor cell membrane-modified polydopamine (PDA)-$CaCO_3$ NPs (CPCaNPs).[71] Once the CPCaNPs targeted and penetrated deep into the acidic TME, the CO_2 bubbles generated by the decomposition of CPCaNPs significantly enhanced the US signal. Additionally, the PDA of CPCaNPs could generate PA signals and perform effective PA therapy in primary tumors. Furthermore, when these nanoprobes were combined with an immune checkpoint pathway blockade, tumor recurrence and migration were effectively inhibited. However, the endocytosis of lysosomes, can lead to the drug being phagocytized and cleared before reaching the designated location, reducing drug delivery efficiency.[84] To address this issue, catalase-driven nanomotors were constructed,[39] with high cellular uptake and lysosomal escape. This system provided a specific mechanism for preventing the proliferation of tumor cells and inducing cell death. The nanomotors were based on the self-determination technology of the $CaCO_3$, PEI, and catalase coat layer. The effect of the PEI proton sponge and the CO_2 produced by the $CaCO_3$ breakthrough helped control the nanometer from the lysosome to the cytoplasm, achieving prolonged retention. Additionally, it was demonstrated that nanomotors could strongly influence the properties of tubulin through paclitaxel and siRNA, leading to programmed cell death in 4T1 tumor cells. Therefore, this system can be used as a safe and vital system of combining drugs for the active treatment of breast cancer. In another work, Janus $CaCO_3$ micromotors (JCPMs) driven by NIR light/gas were demonstrated to overcome multiple physiological barriers for efficient drug delivery.[85] The JCPMs had thermophoretic movements driven by NIR light. They featured precisely controlled start/stop switches, speeds, and motion trajectories in physiological media and autonomous propulsion driven by CO_2 bubbles in acidic environments (Figure 4). Under the conditions caused by the axis, the JCPMs showed multilevel gas and drug release in cells. This strategy could ensure the active substance to practice the therapeutic effects

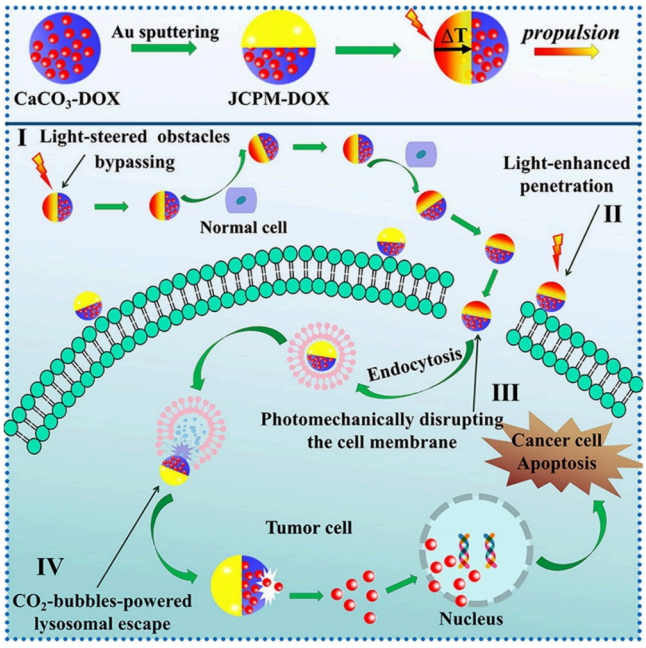

Figure 4. Generation of CO_2 bubbles from CBN. Schematic illustration of the synthesis of the Janus $CaCO_3$ micromotors (JCPMs) and the light/gas cascade-propelled JCPMs to overcome sequential and multistaged biological barriers for precise doxorubicin delivery and efficient tumor cells' killing. Figure reproduced with permission from Elsevier BV,[85] Copyright 2021.

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Table 2. Summary of Typical $CaCO_3$-Based Nanoplatforms (CBN) for Multimodal Cancer Therapy

Nanoformulation	Cancer type	Strategy	ref
$CaCO_3$@CoP	4T1 breast cancer cells	The synergy of CDT and calcium overload to maximize oxidative damage and enhance cancer therapy	92
$CaCO_3$/Cu/Pt (oxali Platin)	4T1 breast cancer cells	Neutralize TME; oxaliplatin is used for chemotherapy; Cu^{2+} is used for CDT; the oxaliplatin and produced ROS could also induce the ICD of cancer cells	93
CDDP/OA-LCC	Hepatocellular carcinoma	Co-delivery of CDDP and OA via a pH-sensitive $CaCO_3$ drug delivery system for enhanced tumor efficacy and reduced adverse effects	24
CPCa	4T1 breast cancer cells	Available for tumor targeting and imaging-guided PTT with synergistic immunotherapy	71
$CaCO_3$/PDA-SA	Melanoma	Enhanced melanoma treatment by the synergistic effect of PTT and Ca^{2+} interference therapy	20
LST-IR820-Ca	Triple negative breast cancer	Synergistic PTT/PDT therapy and exploitation of LST to reduce solid stress of the tumor through depletion of collagen I	94
CuS@Axitinib-SiO_2@2-Deoxy-D-glucose(2-DG)-$CaCO_3$-RGD	4T1 breast cancer cells	Achieve severe tumor starvation and further enhance tumor treatment with the aid of PTT, CDT, and TME improvement	95
$CaCO_3$-GEM-Triapine	Pancreatic cancer	Inhibition of cancer cell proliferation, migration and resistance to GEM using 2D PANC-1/GEM cells and 3D tumor spheroids enhanced the therapeutic efficacy of GEM-based chemotherapy.	96
G/A@$CaCO_3$-PEG	Pancreatic cancer	Increase heat-based tumor therapy by limiting ATP availability	37
IrCOOH-$CaCO_3$@PEG	4T1 breast cancer cells	The combination of calcium overload and Ir complex irradiation with the generation of ROS leads to tumor death	78
CaNPs@Gel-MS	Hepatocellular carcinoma	Reverses lactate-induced chemotherapy resistance during TACE treatment	97
$CaCO_3$@TCL/CpG	Triple negative breast cancer	Neutralize TME and promote immunotherapy	66
O_2-FeCOF@$CaCO_3$@FA	4T1 breast cancer cells	Establishment of a mitochondrial pathway that inhibits tumor growth and synergizes with PDA using dual-activated MPTP channels	98
PGP/$CaCO_3$@IR820/DTX-HA	Castration-resistant prostate cancer	TME-activated synergistic PTT/PDT/chemical therapy strategy	35
IL-12 mRNA@cRGD-CM-$CaCO_3$	Glioblastoma multiforme	Cross the blood-brain barrier and cause necrotic prolapse-induced immune responses and IL-12 mRNA transfection by ultrasound irradiation	62
$CaCO_3$@Cur@QTX125@HA	Colorectal cancer	Specific inhibitory effects on colorectal cancer growth	78,99

selectively on tumors and avoid side effects. The micromotor cascade-driven model designed in this article to traverse biological barriers will improve the delivery efficiency of tumor treatment.

4. MULTIMODAL CANCER THERAPY

It is often challenging to completely eradicate tumors using a single method, and employing multiple modes of therapy can effectively destroy the tumor while reducing side effects.[86,87] CBN can be used for multimodal cancer therapy (Table 2), including chemodynamic therapy (CDT), RT, PTT, PDT, sonodynamic therapy (SDT), chemotherapy, immunotherapy, starvation therapy, and gas therapy.[14,51,71] Due to their excellent drug-loading capability, CBN are well-suited for multimode cancer treatment. External stimulation can activate cell stress reactions (CSR), which counteract the antitumor effect. To address this issue, Liu et al. proposed a cell stress-responsive strategy and developed GOX loaded Cu_3BiS_3 nanosheets (CBSG NSS) wrapped in $CaCO_3$ (CBSG@$CaCO_3$) as a new type of nanoagent to achieve effective combination treatment of starvation therapy and CDT (Figure 5a).[88] The CBSG@$CaCO_3$ not only induces external stimuli, such as energy consumption and oxidation stimulation, but also disrupts the CSR mechanism. After the nanoagent enters cancer cells, the outer layer of $CaCO_3$ breaks down and releases CBSG NS, which possesses intrinsic photothermal properties, accelerating external irritation under NIR-II laser irradiation. Meanwhile, $CaCO_3$ can block CSR by suppressing the expression of P27 and NRF2, thereby destroying the adaptive survivability of cancer cells. Moreover, a multimodal Ca^{2+} nanoregulator has been developed for the first time, which combines PTT with a mitochondrial Ca^{2+} overload strategy to inhibit tumor development.[89] The synergistic Ca^{2+} nanoregulator SA/Cur@$CaCO_3$−ICG(SCCI), was prepared by cross-linking sodium alginate (SA) onto the surface of $CaCO_3$ NPs coated with conductive adhesive. SCCI has been shown to enhance photostability, produce a large amount of Ca^{2+} at low pH, decrease mitochondrial membrane potential, and downregulate ATP production. This SCCI has demonstrated good targeting, biocompatibility, and antitumor effects, significantly inhibiting tumor cell proliferation and producing a direct killing effect. This therapeutic strategy, based on ion interference and PTT, holds great therapeutic potential and provides a new perspective for antitumor therapy.

The regulation of ROS in tumor therapy has been affected by the inherent characteristics of the TME, such as GSH excessive expression, hypoxia, and limited H_2O_2 efficiency. In order to utilize ROS to promote tumor treatment, Zhao et al. have established intelligent copper-dropped $CaCO_3$-loading acoustic agents Ce6 NPs (Cu/$CaCO_3$@Ce6, CCC NPs), and realized the combination treatment of calcium overload enhanced CDT and SDT (Figure 5b-e).[90] In the TME with weak acidity and high GSH, CCC NPs released Ca^{2+}, Cu^{2+} and Ce6. The release of Cu^{2+} could not only consume GSH and convert it to Cu^+ through oxidation reactions but also provide hydroxyl radicals (·OH) that could generate CDT by a Fenton-like reaction. Under ultrasound treatment, the oxidation inside the cell could be greatly enlarged by the outbreak of singlet oxygen from SDT. In addition, Ca^{2+} internal flow exacerbated the destruction of mitochondria, which further accelerated the oxidation level. The simple and feasible design of the nanoregulator based on $CaCO_3$ will be further developed as examples of ROS-regulated cancer treatment.

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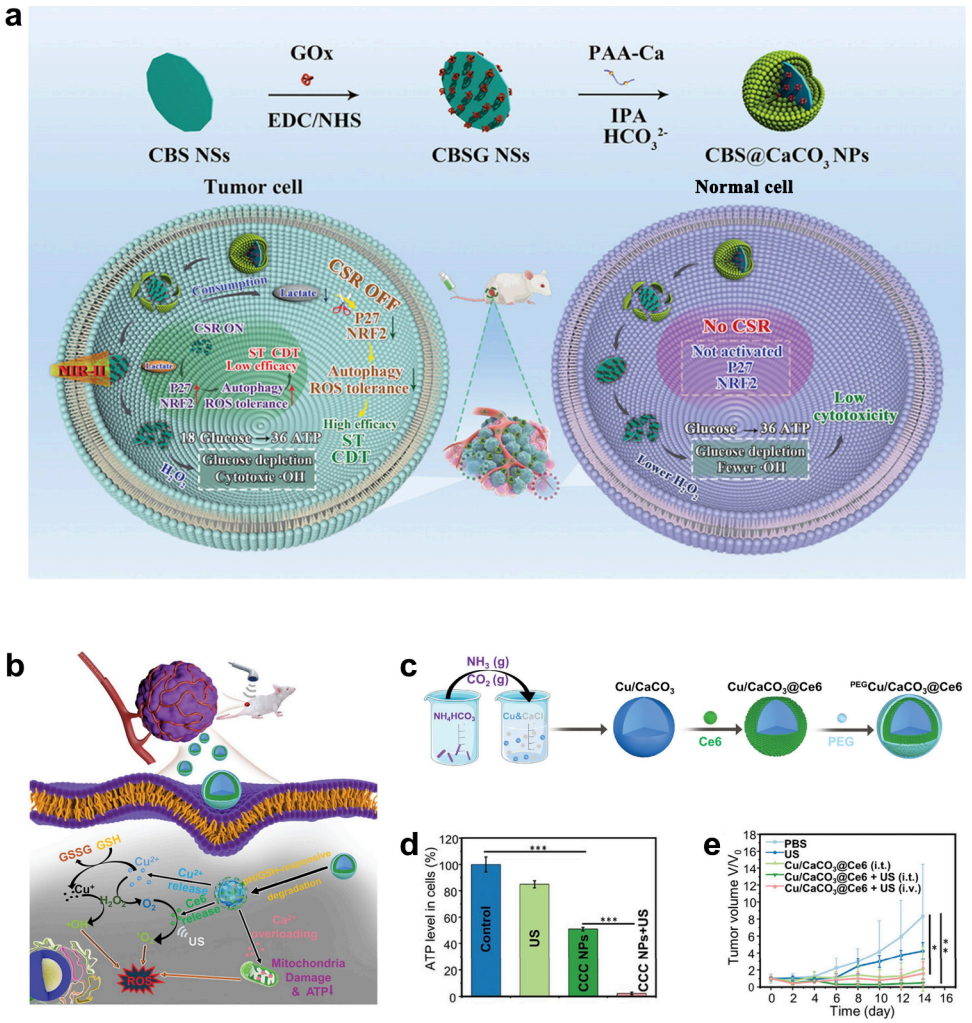

Figure 5. Multimodal cancer therapy based on CBN. (a) The illustration of the synthesis of degradable CBSG@$CaCO_3$ NPs and intracellular therapeutic mechanisms of improving drug-free cancer therapy by interdicting the cellular stress response (CSR) or not. Figure reproduced with permission from Wiley-VCH GmbH,[88] Copyright 2023. (b) Schematic illustration of Cu/$CaCO_3$@Ce6 NPs-dominated advanced cancer therapy via multiple ROS amplification. (c) Schematic illustration of the synthesis of PEG-decorated Cu/$CaCO_3$@Ce6 NPs. (d) Relative intracellular ATP levels of 4T1 with different treatments. (e) Tumor volume changes of various groups with different treatments. Figure reproduced with permission from Wiley-VCH Gmb,[90] Copyright 2022.

Additionally, pyroptosis offers new prospects for cancer immunotherapy. Novel pyroptosis inducers based on CBN have already been developed for cancer immunotherapy. Zheng et al. designed biodegradable Ca^{2+} nanomodulators (CaNMs) as Pyroptosis inducers for cancer immunotherapy via mitochondrial Ca^{2+} overload.[91] The obtained CaNMs can decompose and release Ca^{2+} and curcumin at low pH, causing a sudden surge of mitochondrial Ca^{2+} ions and ultimately leading to Pyroptosis. This work confirmed for the first time the occurrence of Pyroptosis triggered by mitochondrial Ca^{2+} overload and also demonstrated a strong immune response generated by CaNMs, as well as significant inhibition of tumor proliferation and lung metastasis. This work will provide new inspiration for tumor immunotherapy based on Ca^{2+} nanomodulators.

5. CONCLUSIONS, CHALLENGES AND FUTURE BREAKTHROUGHS

CBN is a type of novel nanomaterial and has been widely used in the biomedical field. In recent years, the application of CBN in antitumor research has been widely investigated. However, there are still some problems with their actual antitumor applications mainly in the following aspects.

5.1. Design and Fabrication. Numerous approaches exist for synthesizing CBN, yet their efficacy can be influenced by various elements, including the temperature and duration of the reaction, the presence of surfactants, the type of equipment used, and the specific process parameters, which can restrict their potential applications in cancer therapy.[100,101] If these reaction conditions are not properly controlled, then the morphology and stability of NPs will be affected. For example, the most used gas diffusion method to prepare CBN still has some shortcomings. Due to the slow reaction speed of the gas diffusion method, and the need for fine control of the reaction conditions, its production efficiency is low and the yield is relatively limited.[102] When the gas diffusion method is used to prepare CBN, the particle size distribution is often uneven, which leads to an unstable quality of the product. This method requires the use of many organic solvents in the preparation process, which not only increases the cost but also causes pollution to the environment. Thus, in the future, it is still necessary to design accurate methods to control the size,

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composition, and surface modification to greenly synthesize large quantities of CBN.

5.2. Therapeutic Mechanism. The therapeutic effect of CBN on a tumor can be affected by many factors. For example, the particle size, surface properties, concentration, and other factors of CBN will affect its antitumor effect. The process and mechanism of the antitumor effect of CBN still need to be explored. It is necessary to study the pharmacokinetics and pharmacodynamics of CBN to understand its transport processes in vivo. For example, the transport of CBN to target organs and tissues needs to be studied. First, to break through the dynamic barrier, nanomaterials need to be stable and circulate in the bloodstream for a long time in the presence of multiple enzymes, proteins, and immune cells. Although PEGylating technology can effectively extend the cycle time to a certain extent, it still cannot effectively reduce the uptake of nanocarriers by reticuloendothelial systems.[88,103] Second, CBN is depolymerized or degraded at desired action sites such as weakly acidic TME or within endosomes and lysosomes. The NPs taken up by cells are required to disintegrate and release their cargo to achieve good therapeutic effect outside or inside the cell. Thus, ideal CBN should be able to load enough biologics and release them at the target site in a sustained manner. Finally, the type and stage of the tumor will also affect the therapeutic effect of CBN. Therefore, it is necessary to further study the mechanism of action of CBN to understand how it acts on tumor cells to optimize treatment.

5.3. Biocompatibility/Biosafety. The biosafety of CBN has not been fully verified.[104,105] Although some studies have shown that CBN has an effective killing effect on tumor cells, its effect on normal cells still needs further study. Although calcium is an essential element for the human body, excess calcium can lead to blood clots, hypercalcemia, and other potential dangers.[106] In addition, the metabolism and excretion pathways of CBN in the body are unclear and may cause potential biological risks.[107] It is still necessary to study the characteristics of absorption, distribution, biotransformation, and excretion of CBN in vivo, as well as its influencing factors and mechanism. Recent studies have assessed short-term toxicity in mice only by demonstrating immune response and organ injury after injection of CBN, which is clearly insufficient for biosafety assessment.[108] For the clinical transformation of CBN, it is worth systematically studying the long-term effects of CBN using multiple models.

In summary, there are still some challenges to be overcome in CBN antitumor applications. In future studies, more attention should be paid to the biosafety, metabolic pathways, and factors affecting the therapeutic effect of CBN to provide a more reliable basis for designing CBN for tumor treatment.

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