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REVIEW

Unlocking the potential of amorphous calcium carbonate: A star ascending in the realm of biomedical application

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Abstract Calcium-based biomaterials have been intensively studied in the field of drug delivery owing to their excellent biocompatibility and biodegradability. Calcium-based materials can also deliver contrast agents, which can enhance real-time imaging and exert a Ca^{2+}-interfering therapeutic effect. Based on these characteristics, amorphous calcium carbonate (ACC), as a brunch of calcium-based biomaterials, has the potential to become a widely used biomaterial. Highly functional ACC can be either discovered in natural organisms or obtained by chemical synthesis However, the standalone presence of ACC is unstable *in vivo*. Additives are required to be used as stabilizers or core−shell structures formed by permeable layers or lipids with modified molecules constructed to maintain the stability of ACC until the ACC carrier reaches its destination. ACC has high chemical instability and can produce biocompatible products when exposed to an acidic condition *in vivo,* such as Ca^{2+} with an immune-regulating ability and CO_2 with an imaging-enhancing ability. Owing to these characteristics, ACC has been studied for self-sacrificing templates of carrier construction, targeted delivery of oncology drugs, immunomodulation, tumor imaging, tissue engineering, and calcium supplementation. Emphasis in this paper has been placed on the origin, structural features, and multiple applications of ACC. Meanwhile, ACC faces many challenges in clinical translation, and long-term basic research is required to overcome these challenges. We hope that this study will contribute to future innovative research on ACC.

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1. Introduction

Nanostructured biomaterials hold a vital position in the field of drug delivery applications due to their nanoscale, targeted delivery, and biocompatibility[1,2]. The rise of nanotechnology platforms has provided enormous benefits for designing functionalized drug carriers and enabling targeted drug delivery[3–5]. Nanotechnology, involving inorganic materials, is an important part of biomedical engineering and has multiple therapeutic functions, including imaging and targeting[6–9]. Although many inorganic nanomaterials with biocompatible properties have been developed for medical diagnosis and treatment, many of the available formulations contain potentially toxic elements[10]. Calcium-based biomaterials, an inorganic biomaterial, are promising for drug delivery applications due to their excellent biocompatibility, biodegradability, and lack of significant toxicity[11–13].

Calcium-based biomaterials contain calcium, which is present in the human body, and calcium ion (Ca^{2+}) plays an indispensable role in regulating cellular signaling pathways[14,15]. Calcium carbonate ($CaCO_3$) is an abundant inorganic biomaterial mineral in biomineralization that plays an essential role in life sciences and has been used in regenerative medicine, drug delivery, and a wide range of personal care products[16–21]. Especially, it exhibits extremely high encapsulation and release rates in the delivery of antineoplastic drugs[22]. $CaCO_3$ has six different crystalline forms (amorphous calcium carbonate, mono-hydrocalcite, calcium carbonate hexahydrate, vaterite, aragonite, and calcite), with calcite being the most thermodynamically stable form, and the least stable form being amorphous calcium carbonate (ACC)[13,23]. ACC has various advantages, such as nanometer size, porosity, and biocompatibility. However, its thermodynamic instability hinders its widespread use in drug delivery systems[24–28].

ACC has no contain heavy metals and has been demonstrated to be non-cytotoxic in numerous studies. ACC is of both biological and chemical origin and has shown good biosafety, excellent structural characteristics, and responsiveness to stimulation, which have led many researchers to pursue the use of ACC in biomedical applications. To provide a reference for further research of ACC in the pharmaceutical field, we summarized the stabilization strategies of ACC and its multiple applications.

2. Origin and structural characteristics

2.1. Biological origin

Biogenic ACC was first identified in the early 20th century[29]. The existence of the amorphous state in organisms has been demonstrated, and stable ACC has been found in the exoskeletons of crustaceans, the intestinal lumen of the gilt-head seabream, and crayfish gastroliths[30–33]. As mentioned above, ACC is considered a transient precursor in the formation of $CaCO_3$ biominerals, which crystallizes into more thermodynamically stable calcite crystals in the presence of moisture. Thus, the existence of the amorphous state in organisms suggests a unique stabilization mechanism for ACC in organisms[23,30,34,35]. Because of thermodynamic instability, ACC is rarely found in nature and occurs in only a few organisms. Biological substrates that are important for ACC stability have been identified by analyzing the substances that coexist with ACC in organisms[36]. In-depth studies of substances that coexist with stable ACC in living organisms can not only provide a deeper understanding of the stabilization mechanism of ACC but also promote the development of methods for preparing stable ACC *in vitro* and biomedical applications[37]. For instance, crayfish gastroliths contain stable amorphous states, and small molecules such as phosphate citrate have been found among the coexisting materials, which may be participating in the stabilization mechanism of ACC[36]. Presently, the stabilizing effect of these molecules has indeed been demonstrated[38–41].

2.2. Chemical synthesis

Relevant articles have been published showing that scientists tend to use chemical synthesis to obtain stable ACC and use it for research in biomedical applications, as shown in Table 1[42–47]. The most common method of chemical synthesis in the ACC is gas diffusion[42,43] (Fig. 1A). Gas diffusion is a simple method for synthesizing stable monodisperse ACC nanoparticles in organic media[42,43]. $CaCl_2$ was dissolved in ethanol as a source of Ca^{2+}, and NH_4HCO_3 or $(NH_4)_2CO_3$ decomposed to produce CO_2, which reacted with Ca^{2+}. Finally, we obtained dry and stable nanoscale ACC after the removal of solvent[42,48–52]. However, currently, this method is mostly suitable for small-scale experiments. Empirical data based on measurements of the final product's mass has indicated that the gas dispersion method can achieve a yield of up to 95%[52]. In addition to the gas diffusion method, a variety of other chemical synthesis methods have been developed. The aerosol-based synthesis method relies on the rapid reaction of calcium hydroxide aerosol with CO_2, followed by rapid drying using a diffusion dryer to prevent crystallization[44] (Fig. 1B). This method offers good reproducibility and reduces the use of organic solvents, but it requires more advanced equipment. The present investigation utilizes mixed-flow reactor methods that rely on the seamless integration of equipment. The feedstock is delivered *via* syringe injection to the opposing sides of the base of the reactor unit. The reactor is consistently agitated and stirred. Resultantly, the ACC content reaches a state of supersaturation, subsequently leading to precipitation. The ACC solution suspension is then channeled out of the reactor through an outflow tube located at the top section, and ultimately captured in a vacuum flask equipped with a 0.20 mm nylon filter[45] (Fig. 1C). This method is suitable for experiments with high production demands but requires the involvement of stabilizers. Meanwhile, a filtration freeze-drying method in aqueous solutions has been successively developed[46,47](Fig. 1D). Additive-free and physisorption-free ACC was synthesized by it. Overall, each of the methods exhibits distinct characteristics. Hence, when selecting a synthesis method, it is crucial to consider factors such as production yield requirements, laboratory infrastructure, and cost considerations. Moreover, it is important to note that the field of ACC synthesis

Table 1 Different methods of chemical synthesis of ACC.

Method	Advantage	Disadvantage	Reaction principle	Ref.
Gas diffusion method	Easy operation; low cost	Poor reproducibility; limited amount	$CaCl_{2(aq)} + NH_4HCO_{3(s)} \rightarrow CaCO_{3(s)} + NH_4Cl_{(aq)} + HCL_{(aq)}$	42,43
Aerosol-based synthesis method	Reproducibility; impurity-free; organic solvents-free	Complex reaction devices	$CaCl_{2(aq)} + NaHCO_{3(aq)} \rightarrow CaCO_{3(s)} + NaCl_{(aq)} + CO_{2(g)} + H_2O_{(l)}$	44
Mixed flow reactor method	Controllable conditions; large amounts	Dependence on stabilizers	$Ca(OH)_{2(aq)} + CO_{2(g)} \rightarrow CaCO_{3(s)} + H_2O_{(l)}$	45
Filtration-freeze drying method	Reproducible; extended atmospheric stability; physisorbed-free	Stringent vacuum requirement	$CaCl_{2(aq)} + Na_2CO_{3(aq)} \rightarrow CaCO_{3(s)} + NaCl_{(aq)}$	46,47

aq: aqueous; s: solid; g: gas; l: liquid.

methods is a dynamic one, with ongoing research and the possibility of novel approaches and advancements in the future.

Ultimately, the key to chemical synthesis lies in the selection of suitable reactants that provide Ca^{2+} and CO_3^{2-} and in preventing the reactions from occurring in high heat and humidity environments for longer times. The instability of ACC in environments with high heat and humidity often results in accelerated crystallization reactions, as suggested by various studies[30,53,54]. Consequently, almost all synthesis methodologies involve the incorporation of organic solvents and additives to curb these destabilization effects. Notably, there is a need to reduce the use of organic solvents, including ethanol, and additives during ACC nanoparticle synthesis to encourage environmentally friendly chemistry[55]. The synthesis process is considerably delicate and prone to variations in the size and shape of the ACC particles due to extrinsic factors such as adverse changes in temperature, reaction mixing rate, and impurities in reaction precursors[25]. An analysis of the synthesized product was achieved through spectroscopy and diffraction to ascertain its characteristic absorption peaks, which aided in the identification of the desired product and assessment of its purity[56].

2.3. *Characteristics*

2.3.1. *Characteristics of ACC*

The biocompatibility and biodegradability of a substance are recognized as crucial factors when evaluating its suitability for drug delivery systems[57]. Of late, $CaCO_3$-based carriers have been

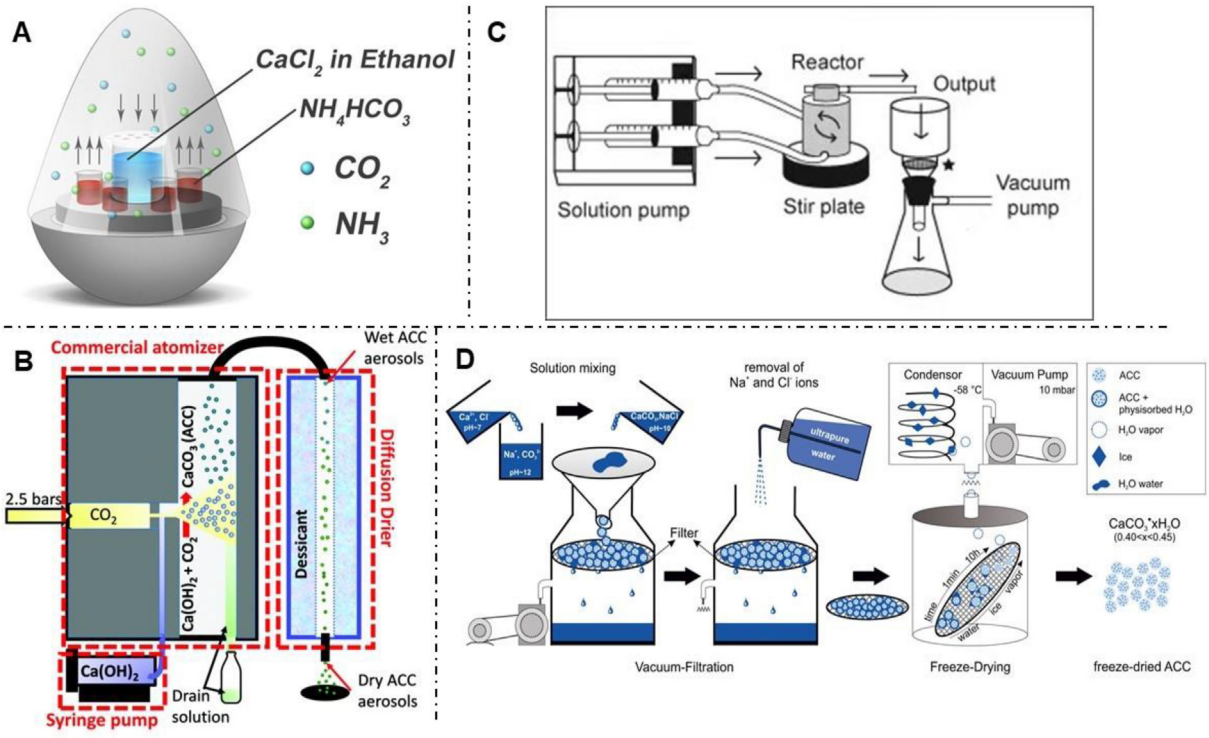

Figure 1 Chemical synthesis methods of ACC. (A) Gas diffusion method. (B) Aerosol-based synthesis method. Reproduced with the permission from Ref. 44. Copyright © 2019 The Royal Society of Chemistry. (C) Mixed flow reactor method. Reprinted with the permission from Ref. 45. Copyright © 2013 Elsevier Inc. (D) Filtration-freeze drying method. Reprinted with the permission from Ref. 46 Copyright © 2016, American Chemical Society.

identified to exhibit biodegradability and safe renal excretion[25]. The acid sensitivity of $CaCO_3$ allows for the chemical release of drugs in low-pH environments[58]. Furthermore, $CaCO_3$ materials are easily synthesized, low-cost, and accessible[26]. Based on the existing advantages of the $CaCO_3$ structure, ACC also has superior characteristics to other crystalline forms, thereby enhancing the possibilities for biomedical applications. The shape of ACC is irregular, and its density and hardness are not significantly different from those of the crystals[30]. Notably, ACC is composed of tiny clusters of approximately 2 nm as revealed using cryo-transmission electron microscopy[59,60] (Fig. 2). The aggregation of individual ACC particles rather than physically fused agglomerates to form larger nanoclusters with porous properties and high specific surface areas can improve drug delivery and accelerate drug release[24]. The solubility of $CaCO_3$ varies, depending on the water molecules present in its structure[53]. Experimental investigation reveals that the ACC structure has the highest solubility as it carries the least amount of water, which is advantageous for drug bioavailability[53,61].

In addition to the above-mentioned advantages of ACC, water instability is considered a barrier to its use in biomedical applications. Despite concerns about the water instability of ACC hindering its biomedical application, surface modification and additive use offer plausible strategies to overcome this obstacle[62]. The lock/unlock technique, for instance, employs a protective shell to avoid water destabilization, degrades at the targeted site, and transforms the hurdle into an asset for effective drug release from the drug delivery system[62].

2.3.2. *Characteristics of ACC decomposition products*

ACC breaks down to generate Ca^{2+} and CO_2 when exposed to acid. Ca^{2+} is an important signaling molecule within cells, while CO_2 microbubbles improve the contrast of ultrasound (US) imaging[63]. Due to these properties, ACC has the potential to be a valuable and biocompatible contrast agent carrier for diagnostic US imaging. Additionally, it has been shown that microbubbles can "explode" under US irradiation, leading to tumor growth inhibition and tumor cell necrosis[64]. Monitoring tumor accumulation and targeted antitumor therapy is facilitated by the production of bubbles in acidic tumor environments. Ca^{2+} is involved in regulating cellular mechanisms and pathways; it activates inflammation-related signaling pathways, silences programmed cell death-ligand 1, and enhances T-cell sensitivity to exogenous antigens[65–67]. Simultaneously, Ca^{2+} can induce significant "Ca^{2+} interference" and an imbalance in Ca^{2+} homeostasis within cells and organelles. This interference induces ER stress in cancer cells and promotes the production of reactive oxygen species, ultimately leading to the release of damage-associated molecular patterns (DAMPs) and an immunomodulatory effect[68–70]. This platform has shown to be effective in treating primary and metastatic tumors with minimal side effects[70].

3. Crystallization transformation and stabilization strategies for ACC

3.1. *Crystallization transformation*

Utilizing cryoelectron microscopy, scientific investigations have provided empirical evidence to substantiate that the process of crystalline $CaCO_3$ formation commences with the formation of prenucleation clusters. The agglomeration of these clusters engenders the nucleation of amorphous nanoparticles within the solution, thereby instigating the process of crystallization[71]. The progression of crystalline transformation entails the dehydration of the stable ACC and subsequent crystallization after dissolution[24,41,56,72]. Experimental demonstrations have ascertained that stable ACC incorporates a scant quantity of structural water and undergoes a highly precarious transition to a transient ACC state upon dehydration[29]. Dehydration reactions are heat absorbing, and the adsorbed water on the surface of ACC reaches a critical level

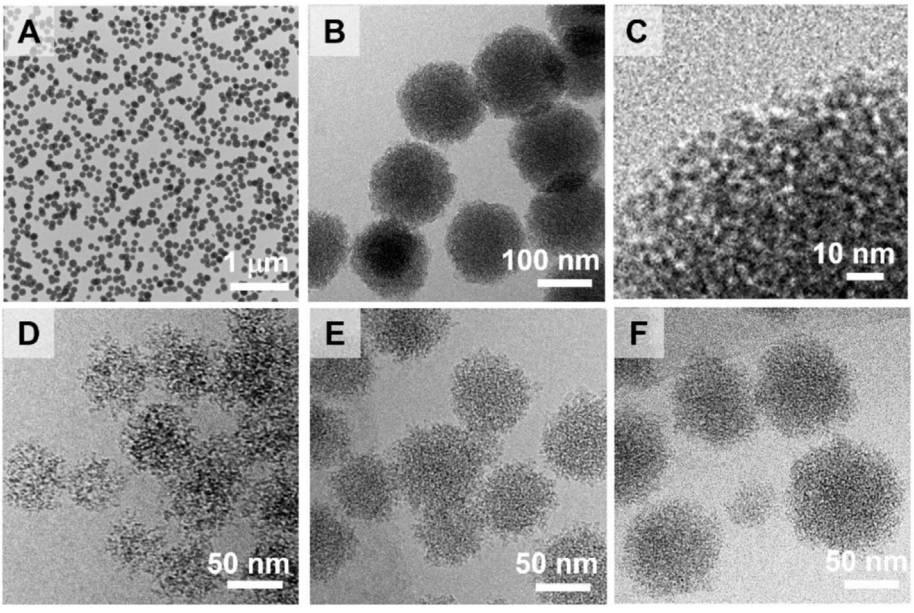

Figure 2 Structure of ACC nanospheres. (A) Transmission electron microscopy images of ACC nanospheres synthesized by gas diffusion. (B and C) Cryo-TEM images of ACC nanospheres composed of clusters. (D−F) Cryo-TEM images of ACC nanospheres during growth at different times. Reprinted with the permission from Ref. 59. Copyright © 2022 Wiley-VCH GmbH.

that facilitates the dissolution of ACC. Consequently, the application of thermal energy and elevated humidity expedites the crystallization process of ACC[30,46,53]. The stability of ACC is mainly controlled by the internal structural dynamics in conjunction with the water content[73]. The physical adsorption of H_2O at critical thresholds triggers the transformation of ACC to crystalline $CaCO_3$[46]. Accordingly, the establishment of a protective shell on the surface of ACC serves to mitigate water adsorption and, in turn, contributes to the preservation of ACC stability. In parallel, the introduction of specific additives can impede the crystalline transformation of ACC, either by their incorporation within the ACC structure or by their adsorption onto the ACC surface. Consequently, two stabilization mechanisms are summarized: the utilization of additives and the construction of a core−shell structure[29].

3.2. *Stabilization strategies*

3.2.1. *Additives*

The incorporation of additives as a means to achieve stable ACC nanoparticles during chemical synthesis is widely acknowledged and accepted[74]. Previous investigations have demonstrated that ACC devoid of additives undergoes conversion to crystalline calcium carbonate within a span of 1−4 days in an exposed condition at room temperature[46]. Analyses of ACC derived from biological sources, such as crayfish gastroliths, have revealed the presence of distinct growth layers containing varying levels of inorganic phosphate, citrate, and other small molecules[18]. Notably, highly phosphorylated organic substrates obtained from crayfish ascites have been found to induce the formation of stable ACC *in vitro*. The inclusion of phosphorylated phosphoprotein residues emerges as a key component that assumes a stabilizing role[75]. These findings imply that the presence of multiple stabilizing substances is necessary to uphold the stability of biologically derived ACC and the imitation of natural phenomena can serve as a source of inspiration for the invention of new biogenic materials[36,76].

A comprehensive examination of known additives reveals that they can be broadly categorized into small-molecule and large-molecule additives based on their structural characteristics. Small-molecule additives employ a general mechanism whereby they permeate the interior of the ACC structure, mitigating ion mobility through ion binding and bestowing kinetic stability upon the ACC[73]. During early detection, ACC in a mixture of tricalcium phosphate and ACC was relatively stable[30]. This led to the revelation that small phosphate ion-class molecules can be used as additives to confer stability upon ACC[77,78]. The common small-molecule additive, Mg^{2+}, exerts a stabilizing effect on ACC and influences the mechanism of late-stage ACC crystallization[79−81]. Even at low concentrations, Mg^{2+} curtails the rate of crystallization[82]. As previously mentioned, ACC undergoes a dehydration reaction before crystallization, and Mg^{2+} has a higher hydration energy in comparison to Ca^{2+}[77]. This difference in dehydration energy between Mg^{2+} and Ca^{2+} is believed to underlie the inhibition of ACC precipitation in the presence of Mg ions within an aqueous medium. Simultaneously, the incorporation of Mg^{2+} at crystal growth sites synergistically impedes crystallization[83]. However, excessively high concentrations of Mg^{2+} relative to Ca^{2+} can give rise to a peculiar crystallization process known as direct leapfrog crystallization[84]. Another ACC-stabilizing additive, silicate, exploits its tetrahedral structure to impede the regular accumulation of carbonate. The presence of tetrahedral silicate ions bearing four negative charges may disrupt charge balance and impede ACC crystallization[85]. Adenosine triphosphate (ATP), the cell's energy "currency," can inhibit crystallization in aqueous solutions by poisoning nonhomogeneous nucleation sites and binding to nuclei, so it can also be used as a stabilizer for long-term stabilization of ACC in aqueous solutions[38,86]. In addition, numerous small-molecule additives have been found to be beneficial in maintaining ACC stability, such as citric acid, phytic acid and the metal ion Sr^{2+}, which operate *via* mechanisms similar to that of Mg^{2+}[33,41,54,87].

The coverage of ACC nanoparticles on the surface by macromolecule additives assumes a pivotal in the physical properties and stability of the ACC nanoparticles. The polymer can influence crystallization by binding to the surface of the growing crystals, effectively impeding the precipitation process[16]. For instance, poly(sodium 4-styrene sulfonate) (PSS) serves to stabilize hollow ACC structures, and its presence correlates with a reduction in the mean size of ACC particles. Notably, the size of induced ACC particles escalates with decreasing amounts of PSS in the reaction solution[88]. Another stabilizing additive, poly aspartic acid (pAsp), shares similarities with PSS and modulates nanoparticle size by varying its concentration[89]. In addition, different polymer chain lengths exhibit varying efficacies in stabilizing ACC, with pAsp-200 demonstrating greater efficiency than pAsp-30[90]. Xu et al.[91] developed an *in situ* hydrogel platform which involved the dispersion of pAsp-stabilized ACC nanoparticles in a drug-loaded sodium alginate solution, where the consumption of ACC in the gastric environment triggered the cross-linking of sodium alginate to achieve sustained drug release. Carboxylic acid polymers containing carboxyl groups can be used to modulate the physical properties of ACC, including porosity and storage stability[40]. Nanofibers enable the long-term stability of ACC in high-density nanofibrous collagen gels, and the availability of nanofibrous amphiphilic surfaces is key to this process[92].

In summary, the stability of ACC is effectively controlled by additives through two distinct mechanisms: surface adsorption and intrinsic incorporation. The action mechanism varies between large and small molecules, with large molecules adsorbing onto the surface of ACC nanoparticles, while small-molecule ions exhibit effective stabilization only when they are incorporated into the mineral structure. Large molecules play a crucial role in inhibiting calcite nucleation on the surface of ACC particles, whereas small-molecule ions influence ion mobility, thereby impeding crystal growth[39]. At the same time, the formation of ACC is regulated by the concentration of additives, such as Mg^{2+}[93]. The stability of SiO_2 in ACC is limited by the pH, with a higher pH being more beneficial for preventing crystallization[94]. Temperature elevation weakens the inhibitory effect of Mg^{2+} on ACC crystallization, while the inhibitory effect of sulfate on crystallization remains unaffected by temperature changes[95]. The presence of additives influences the drug-release behavior of drug delivery systems[96]. Hence, the effect of additives on applications should be considered when selecting additives.

3.2.2. *Core−shell structure*

To safeguard ACC from the effects of aqueous conditions, a protective shell with biocompatible and water-resistant properties has been designed (Fig. 3). This protective shell serves the purpose of enhancing the stability of ACC both *in vitro* and *in vivo*[62].

The utilization of lipid-coated ACC offers notable advantages, including facilitated cell entry and the potential for enhanced targeting of the entire drug delivery system through modifications

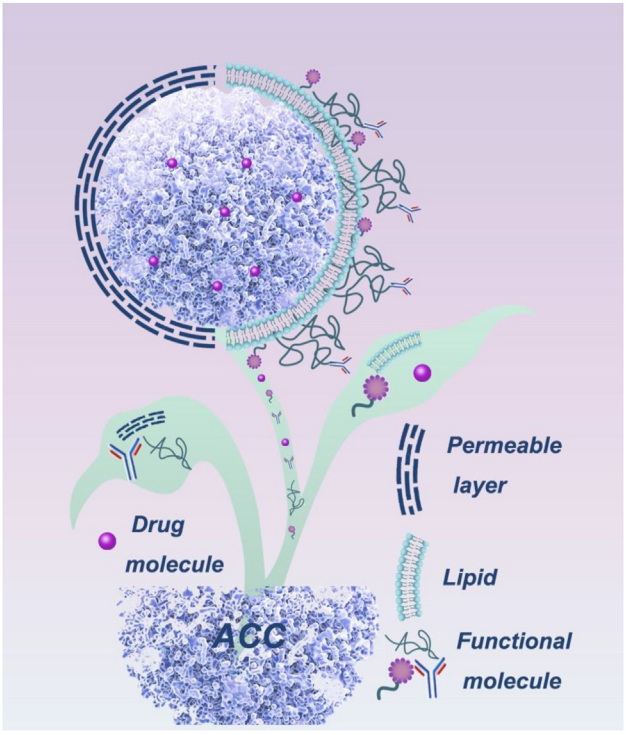

Figure 3 Two different core−shell structures & multi-stage assembly process.

of the lipid layer[97]. The presence of a lipid layer on ACC nanoparticles has been observed to impede the free diffusion efficiency of water molecules[98−101]. If incorporating polyethylene lipids, the glycol chains extend outward from the liposome surface, forming a hydrated layer around the membrane[21,102]. This arrangement effectively protects the hydrophobic tails of lipid molecules from the surrounding aqueous condition. In the absence of polyethylene glycol, negatively charged lipids can rapidly self-assemble around ACC nanoparticles in the presence of Ca^{2+}, resulting in a multilayer compression-like arrangement structure that acts as an efficient waterproof protective layer[55]. Meanwhile, water-soluble and flexible polymer molecules can form conformational "clouds" with sufficient density on the surface of liposomes, providing effective spatial protection[103]. Additionally, the polymer coating on the lipid layer surface reduces the rate of interaction between liposomes and blood components by increasing the free energy barrier for various specific and non-specific reactions[104]. Biocompatible lipids can be selected to construct the protective shell, which not only maintains the responsiveness of the inner core ACC to water but also acts as a barrier against drug leakage[51,105]. The lipid layer, due to its similarity to the composition of cell membranes, can facilitate the delivery of drugs inside cells. The lipid layer is disrupted by intracellular enzymes, leading to the release of the free drug.

Unlike liposomes, certain protective shell structures enable complete drug release outside the cell, without the need for cellular internalization. The permeable SiO_2 shell, in combination with the drug core of ACC, forms a typical core−shell nanoreactor structure[106]. The permeable shell allows for an acidic stimulus to trigger the internal drug release response while preventing drug leakage, allowing the drug to exert its therapeutic effect extracellularly[28]. With advances in materials science, hydrophobic coatings have emerged to control the rate of water penetration and are expected to be utilized for ACC drug nucleus delivery while preventing drug leakage[107].

The core−shell structure provides effective protection for the drug nucleus, improves the stability of the drug in the environment, and avoids rapid blood clearance[108]. This protective feature offers a new avenue for the oral administration of protein-based drugs[109]. The process of constructing a core−shell structure is simple and easily implementable, as the addition of a protective shell component during chemical synthesis results in the formation of a core−shell structure[52]. In summary, the core−shell structure enables extracellular and intracellular drug release; however, it is crucial to ensure the biocompatibility and degradability of the shell material to mitigate potential biosafety risks.

4. Applications

4.1. Self-sacrificing templates

In recent years, nano-drug delivery systems have emerged as promising drug carriers, offering advantages such as high drug delivery capacity, controlled release, and tumor targeting capabilities[110,111]. Nanocarriers with hollow interiors have garnered significant attention due to their large loading capacity[112,113]. They have shown great potential in the diagnosis and treatment of many diseases. For instance, an experiment comparing the immune-enhancing effects of nonhollow and hollow nano adjuvants *in vivo* revealed that low-density hollow structures exhibit superior antigen uptake and enhanced anticancer immunity[114]. When employing organic materials as sacrificial templates for the synthesis of hollow materials, their stability is comparatively limited owing to their heightened susceptibility to variations in ionic strength and temperature. Conversely, the fabrication of inorganic hollow nanostructures utilizing inorganic templates exhibits enhanced stability and abundant shapes[115].

Previously, scientists have synthesized porous nanostructures of multiple shapes and microcapsules with hollow internal structures using $CaCO_3$ nanoparticles as inorganic templates[116−121] (Fig. 4A). The implementation of ACC as a sacrificial template facilitates the synthesis of hollow nano drug delivery systems[122−124] (Fig. 4B). Through the process of "sacrifice" *in vivo*, systems effectively enhance the therapeutic efficacy by inducing Ca^{2+}-overloading[122]. Additionally, Dong et al.[125] developed a biomimetic mineralized $CaCO_3$-poly(dopamine) hollow nanoparticle therapeutic platform that allows the simultaneous loading of imaging and therapeutic molecules for imaging-guided cancer therapy. This platform was specifically designed to enhance the efficacy of tumor photodynamic therapy while significantly reducing photosensitivity toxicity. Notably, hollow nanoparticles synthesized using ACC nanoparticles as inorganic templates generally have a porous surface structure, and the release of the encapsulated drugs has a three-peak delayed release characteristic[118,121,126]. In the quest for ultrasmall hollow nanostructures, ACC has emerged as a favorable template. For example, ultrasmall hollow silica nanoparticles have been successfully obtained using ACC as a template and tetraethyl orthosilicate (TEOS) as the silica source, followed by the gentle removal of ACC using hydrochloric acid[127] (Fig. 4C). It is evident that ACC presents benefits for the fabrication of ultrasmall hollow

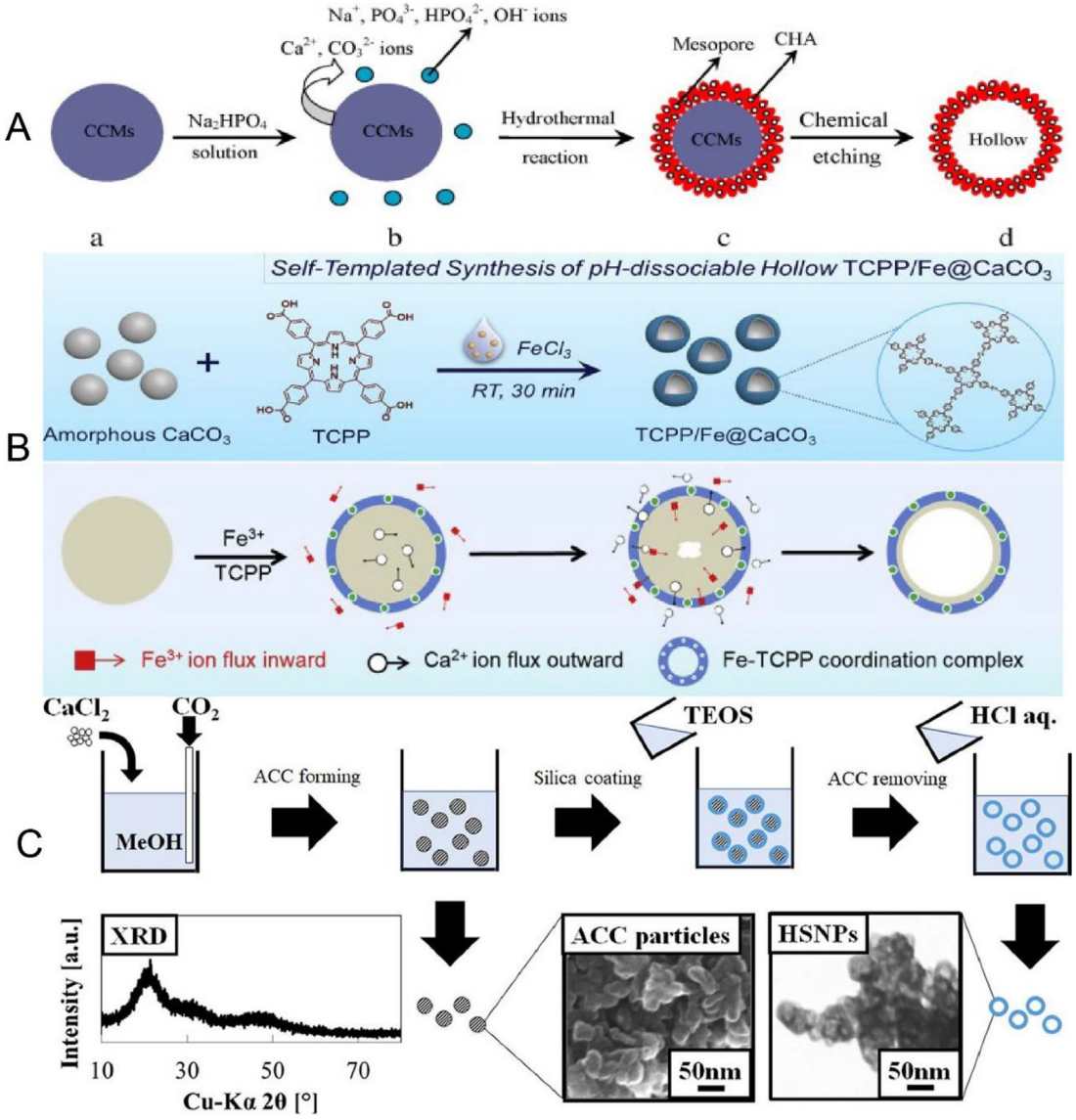

Figure 4 (A) Illustration of the fabrication strategy of HCHAs: CCMs used as sacrificial templates. HCHAs: hollow carbonated hydroxyapatite microspheres with mesoporous structure, CHA: carbonated hydroxyapatite carbonate microspheres, CCMs: calcium carbonate microspheres. Reprinted with the permission from Ref. 121. © 2013 Elsevier B.V. (B) A scheme showing the synthesis process of TCPP-Fe@$CaCO_3$ hollow nanoparticles and the formation mechanism of TCPP-Fe@$CaCO_3$ hollow nanoparticles. TCPP: meso-tetra-(4-carboxyphenyl) porphine. Reprinted with the permission from Ref. 122. Copyright © 2020 Elsevier Inc. (C) Preparation of ultra-small hollow SiO_2 nanoparticles by ACC one-pot method. TEOS: tetraethyl orthosilicate, HSNPs: hollow silica nanoparticles. Reprinted with the permission from Ref. 127. Copyright © 2018 The Society of Powder Technology Japan.

nanostructures owing to its diminutive proportions and its propensity to degrade in the presence of acidic substances.

4.2. *Targeted delivery of antitumor drugs*

Malignancies pose significant threats to human life, and current treatment modalities such as chemotherapy, radiotherapy, and surgery[128,129]. However, they still have limitations, including serious side effects associated with highly potent but toxic chemotherapeutic agents[130]. To address these challenges, functionalized nanocarriers are being explored to reduce drug dispersion in normal tissues. Compared to microscale crystalline $CaCO_3$, the nanoscale structure of ACC enables drug targeting and accumulation at tumor sites through enhanced permeability and retention effects, resulting in prolonged drug retention time and enhanced drug efficacy[24,105,131].

One of the challenges faced by many currently available drug delivery systems (DDSs) is ineffective drug release, where a significant portion of the drug within the carrier fails to convert into an active form at the target site[132]. It has been observed that larger nanoparticles have a higher tendency to extravasate but face difficulties in diffusing through the dense extracellular matrix, which hinders their penetration into tumors[132]. On the other hand, smaller nanoparticles exhibit better tumor penetration but suffer from rapid clearance and short half-life, resulting in insufficient accumulation at the tumor site[133–136]. Both vascular extravasation

and tumor penetration need to be considered when designing tumor-targeting vectors[10,137]. ACC-based delivery systems offer a unique enrichment process that resembles "peeling an onion," enabling multi-stage nano-delivery to ensure deep penetration of nanoparticles[138]. This process allows free drug molecules to penetrate the dense collagen matrix more easily and facilitates continuous intracellular delivery, exerting a powerful proximity effect by gradually penetrating layer by layer[51,137]. Additionally, multi-stage nano-systems can have complementary roles. For example, lipid modifications can enhance the cellular uptake of ACC carriers, but liposomal modifications may lead to rapid uptake by cells of the mononuclear phagocytic system *in vivo*[139,140]. However, the addition of polymer coatings on the outer lipid layer can reduce the interaction rate with blood components, increasing the free energy barrier and prolonging the circulation duration of the delivery system[104]. Subsequently, when the polymer layer is hydrolyzed by enzymes at the target site, the lipid layer can exert its advantageous penetration mode.

The pH value in the tumor microenvironment is lower than that in normal tissues because of increased acid production through glycolysis in tumor cells[141]. The acidic microenvironment is favorable for tumor cell growth and invasion of normal tissues, as well as for inducing apoptosis in normal cells[142]. Conversely, it offers the potential for pH-sensitive ACC to target tumor sites[143]. ACC nanoparticles can react with hydrogen ions in acidic environments, leading to the release of drugs specifically at the tumor site. By consuming H^+, ACC can prevent tumor cell invasion while enabling $CD8^+$ T-cells to improve their responsiveness and restore their tumor-killing activity[144]. In addition, the water instability of ACC serves as a critical property for rapid drug release. These unique properties, including retention, acid sensitivity, and water instability in the tumor environment, make ACC a promising candidate for improving drug delivery and achieving targeted therapeutic outcomes[48,145,146].

Currently, the primary application of ACC is in the targeted delivery of antitumor drugs[147]. However, there is a limitation for most H^+-triggered drug release carriers due to the depletion of H^+, leading to insufficient sustained drug release[148]. To achieve effective antitumor therapeutic effects, it is crucial to design a delivery system that allows specific drug release within the tumor tissue, offers unlimited responsive stimuli, and enhances tissue penetration[51]. Hence, as an abundant stimulus at the tumor site, it would be extremely valuable to endow the ACC with responsiveness to water in a controlled manner[50]. By freeing ACC from stabilizers, it can exhibit high water instability within tumor tissues and explosively release drugs, leading to enhanced tumor penetration and antitumor effects[62,105] (Fig. 5A and B). Biodegradable ACC nanoparticles stabilized with polyacrylic acid have been utilized for pH-responsive drug delivery, resulting in enhanced tumor suppression[96]. In addition to small-molecule chemotherapeutic agents, ACC has also been employed to efficiently deliver small interfering RNAs (siRNAs) for gene therapy[149]. Through functionalized modification of ACC carriers, siRNAs can be specifically transferred to target cells with high transfection efficiency.

However, for drugs that require continuous long-term administration, the burst release generated by ACC-based delivery systems does not satisfy the above therapeutic requirements. To address the problem of sustainable drug release, it has been documented that sodium alginate can effectively overcome the shortcomings of the rapid release of ACC nanoparticles in an acidic environment. When sodium alginate was doped into ACC, gradual sustained drug release behavior was observed at a low rate[150]. The electrostatic interactions between the charged shell layer and protonated drug in the core−shell structure can also generate sustainable drug release. For instance, SiO_2 functions as a protective shell layer for doxorubicin (DOX). Protonated DOX dissociates from the core and then adsorbs onto the negatively charged SiO_2 shell *via* pH-dependent electrostatic attraction rather than direct release, thus allowing for sustained cancer therapy[28]. Long-term chemotherapy can lead to the development of drug-resistant tumor cells, which reduces the sensitivity of these cells to antitumor drugs. Nano $CaCO_3$/DNA coprecipitation has been investigated as a strategy to overcome multidrug resistance at the genetic level. Optimizing the $Ca^{2+}/CO3^{2-}$ ratio enables efficient gene transfection in the presence of serum, offering the potential for effective antitumor therapy by combining gene therapy with chemotherapy through the co-delivery of chemotherapy and gene therapy drugs[151]. The application of ACC has proven to be promising in the administration of chemotherapy drugs along with contrast agents. This approach has demonstrated effective drug release and improved tumor imaging through the use of photodynamic or US stimuli[146,152](Fig. 5C and D).

When the ACC delivery system is in a stable state, it can prevent unwanted drug release from the target cells and effectively avoid the degradation of the drug[152,153]. This nano platform controls the drug "on/off"; that is, it can specifically respond to stimuli at the tumor site, achieving precise drug release at the lesion site, realizing precise drug release at the lesion site, and exhibiting tumor-killing effects comparable to those of free drugs[62,152]. It is also expected to alleviate chemotherapy resistance problems at the genetic level and make drug delivery systems more effective.

4.3. *Regulation of immune function*

Immunogenic cell death (ICD) has emerged as a promising approach to enhance the immunogenicity of tumor cells and trigger robust antitumor immune responses[154]. An imbalance of intracellular Ca^{2+} concentration can lead to endoplasmic reticulum (ER) stress and mitochondrial damage, which play crucial roles in inducing ICD in tumor-synergistic therapy[122,155] (Fig. 6A). Zheng et al.[156] designed a calcium carbonate-based biodegradable Ca^{2+} nano-modulator loaded with curcumin, which induced cell pyroptosis by causing mitochondrial calcium overload under low-pH conditions, thereby activating a powerful immune response for antitumor immunotherapy (Fig. 6B). Certain chemotherapeutic agents, such as oxaliplatin and DOX, have been shown to induce ICD in tumor cells, leading to *in situ* antitumor immunity[157,158]. Tumor cells treated with ICD inducers can induce ER signaling, including the translocation of calreticulin to the surface, the release of high-mobility group protein B1 from the nucleus, and the release of ATP[159]. The expression of these signals enhances immune cell infiltration into tumors and upregulates immune cell activation, acting as an *in situ* vaccine to elicit a systemic immune response against tumor growth[160]. Therefore, as an advantageous calcium-based material, ACC can not only induce ICD on its own but can also be employed as a carrier to deliver ICD inducers, such as adriamycin, which also possesses chemotherapeutic effects[161].

ACC can contribute to the restoration of antigen-presenting cell (APC) viability by depleting acids in the tumor microenvironment while releasing DAMPs from tumor cells. ICD induced by an imbalance of Ca^{2+} concentration can also promote the maturation of the dendritic cells (DCs)[162] (Fig. 6C). Additionally,

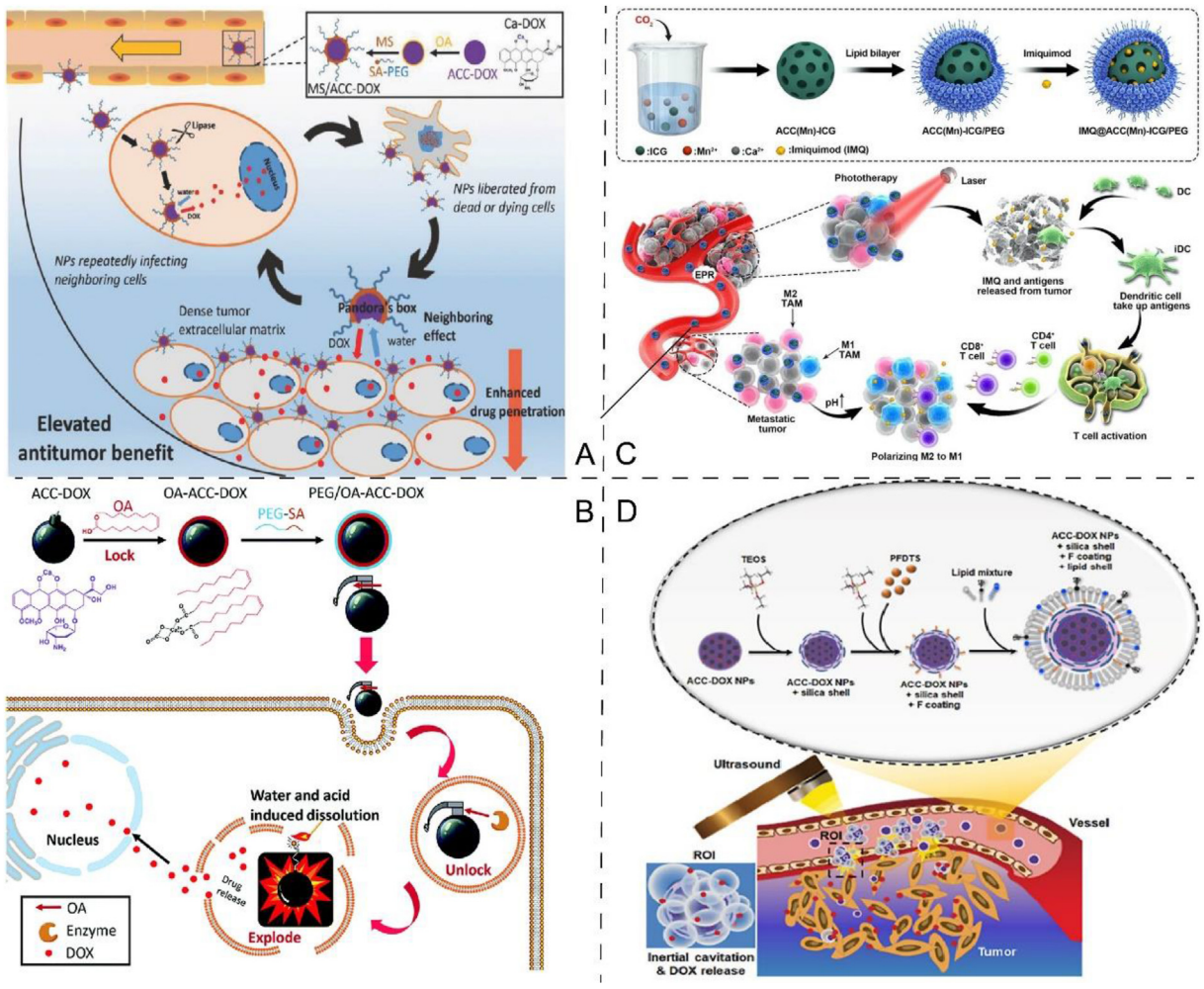

Figure 5 (A) Illustration of formation and elevated antitumor mechanism of "Pandora's box"(MS/ACC−DOX) nanoparticles. MS: monostearin. Reprinted with the permission from Ref. 105. Copyright © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Schematic illustration of the assembly process and disassembly mechanism of PEG/OA-ACC-DOX within cancer cells. PEG: polyethylene glycol OA: oleic acid. Reprinted with the permission from Ref. 62. Copyright © 2017 Royal Society of Chemistry. (C) Scheme of the fabrication of IMQ@ACC(Mn)−ICG/PEG nanoparticles and its photoimmunotherapy process for antitumor therapy. IMQ: imiquimod, ICG: indocyanine green. Reprinted with the permission from Ref. 146. Copyright © 2020 Royal Society of Chemistry. (D) Schematic showing the design of the study. Upon US stimulation, the ADSFL NPs are triggered to undergo inertial cavitation, which generates bubbles, disrupts tumor vessels, and releases DOX into the tumor area. ADSFL NPs: surface fluorinated ACC−DOX silica nanoparticles. US: ultrasound. Reprinted with the permission from Ref. 152. Copyright © 2022, American Chemical Society.

an increase in intracellular Ca^{2+} levels can promote the polarization of macrophages to the M1 type, which can exert antitumor effects[163,164] (Fig. 6D). It is known that to effectively activate cellular immunity, APCs must capture disease-associated antigens and cross-present them to $CD8^+$ T cells *via* major histocompatibility complex class I[165]. ACC, being a calcium-based material, holds the promise that can serve as a carrier for vaccine antigens, facilitate cross-presentation of antigens, and act as an adjuvant[166].

In conclusion, ACC nanoparticles exhibit distinct characteristics in immunomodulation. They effectively neutralize the acidic tumor microenvironment by counteracting the elevated H^+ levels, thereby reversing the immunosuppressive conditions within the microenvironment. Furthermore, ACC nanoparticles have demonstrated the ability to inhibit the polarization of macrophages toward an anti-inflammatory phenotype[146]. In comparison to other immune therapeutic carriers, ACC nanoparticles offer controlled and targeted drug delivery to tumor sites, facilitating the release of therapeutic agents and promoting robust anti-tumor immune responses[167,168]. Additionally, the degradation of ACC nanoparticles generates calcium ions, resulting in disrupted Ca^{2+} homeostasis within tumor cells. With the delivery of drugs, such as DOX, the system synergistically induces immunogenic cell death in tumor cells, thereby exhibiting an in-situ vaccine effect[158,162]. These multifaceted properties of ACC nanoparticles, including their ability to modulate intracellular calcium levels and facilitate targeted drug delivery, are pivotal in enhancing the synergistic effects between chemotherapy and immune-mediated responses.

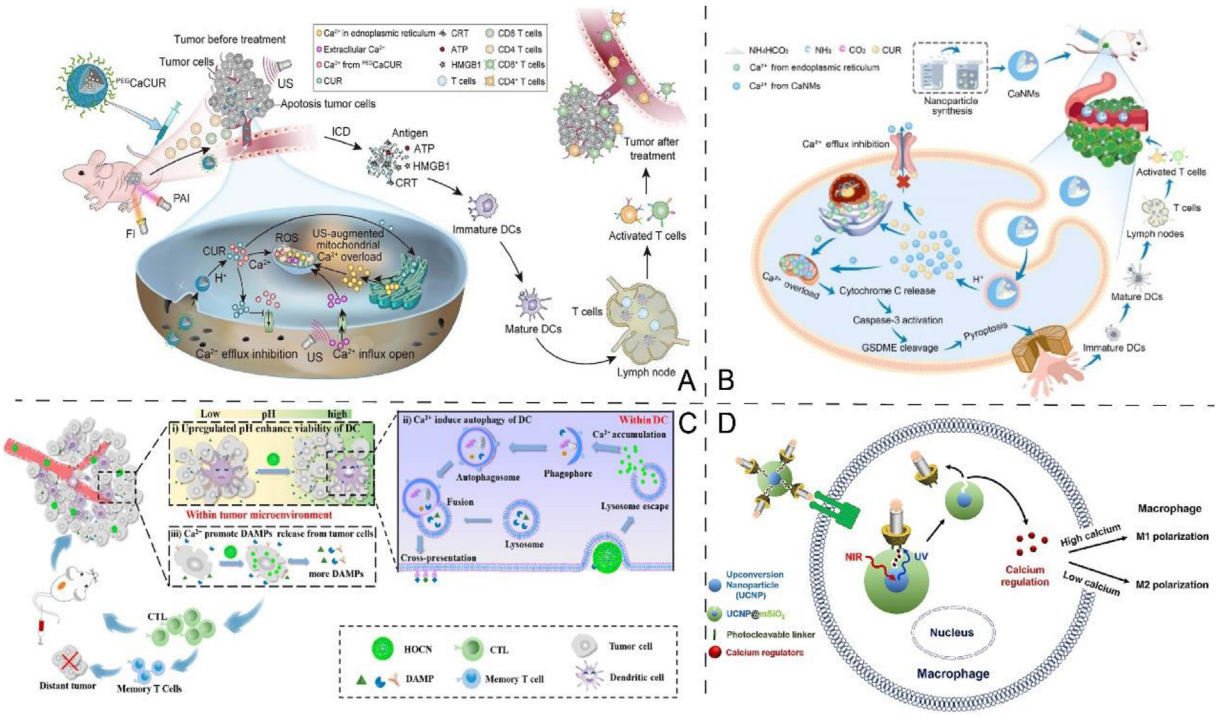

Figure 6 (A) Schematic illustration of acid-sensitive PEG-modified calcium carbonate nanoparticles containing curcumin. Reprinted with the permission from Ref. 155. Copyright © 2021 American Chemical Society. (B) Biodegradable Ca^{2+} nano-modulators as the pyroptosis inducers to activate pyroptosis through mitochondrial Ca^{2+} overload for cancer immunotherapy. Reprinted with the permission from Ref. 156. Copyright © 2022 Wiley-VCH GmbH. (C) Schematic diagram of HOCN disruption of multiple barriers in antigen cross-presentation of DCs for enhanced chemo-immunotherapy, ovalbumin acted as skeleton. HOCN: Honeycomb calcium carbonate nanoparticles. Reprinted with the permission from Ref. 162. Copyright © 2020, American Chemical Society. (D) Scheme of near-infrared light-controlled regulation of intracellular calcium to modulate macrophage polarization. Reprinted with the permission from Ref. 164. Copyright © 2018 Elsevier Ltd.

4.4. *Enhancement of tumor imaging*

Microbubbles can be used as highly sensitive and biocompatible contrast agents for diagnostic US imaging because they have a unique acoustic effect that improves the contrast of US imaging[63]. Current microbubble-based US contrast agents face challenges such as instability in the blood and limited imaging duration[169,170]. Additionally, their micron-sized particle size makes it difficult for them to penetrate the tumor environment through tissue extravasation[171]. Therefore, there is a demand for stable and highly penetrative contrast agents. Calcium-based materials can also deliver contrast agents, such as copper sulfide, which can enhance real-time imaging and exert a Ca^{2+}-interfering therapeutic effect[70]. $CaCO_3$ can react with acid to produce CO_2 bubbles in tumor tissue sites but not in normal tissues, and it can improve the US reflectivity of tumor tissue ultrasonography[172] (Fig. 7A). CO_2 generated at the tumor site can rapidly "explode" under US irradiation, exerting a potent tumor-killing effect[64] (Fig. 7B). Moreover, the synthesis of amorphous composite nanoclusters involving lanthanide gadolinium ions in ACC can reinforce magnetic resonance imaging contrast, offering a promising application in advanced diagnostic technology with low toxicity and scalability[173]. Notably, ACC can be used as a carrier to co-deliver photodynamic and photothermal drugs with fluorescent properties along with chemotherapeutic agents, which can synergistically integrate tumor imaging, chemotherapy, photodynamic therapy, and photothermal therapy to enhance the therapeutic effect. Therefore, ACC is expected to allow for precise and efficient diagnosis and treatment with the aid of the previously mentioned nanoplatforms[169,172].

Collectively, the ACC drug delivery system can perform the dual functions of enhanced US imaging and drug treatment for cancer by triggering both drug release and CO_2 bubble production in an acidic tumor environment, providing an attractive strategy for tumor diagnosis and treatment. Through the utilization of carrier properties and functional modifications, it is possible to design tumor-targeted bioimaging and pH-responsive drug release for integrated diagnosis and treatment[174].

4.5. *Tissue engineering*

Tissue engineering has emerged as a crucial strategy for repairing damaged tissues, particularly in the context of bone repair. Osteoporosis and bone loss have long become a serious public health problem that poses a serious threat to the overall health and quality of life of older adults[175–177]. However, existing clinical approaches for bone reconstruction, whether involving metal alloys and ceramics or autologous and allogeneic bone grafts, have not met the desired outcomes[178,179]. $CaCO_3$ has promising advantages in the treatment of osteoporosis due to its biocompatibility, affinity for natural bone, ease of functionalization, low pH-responsive depolymerization, and inherent bone-repairing properties[180]. Moreover, the field of tissue engineering has seen extensive interest in nanotechnology[181,182]. Consequently, ACC,

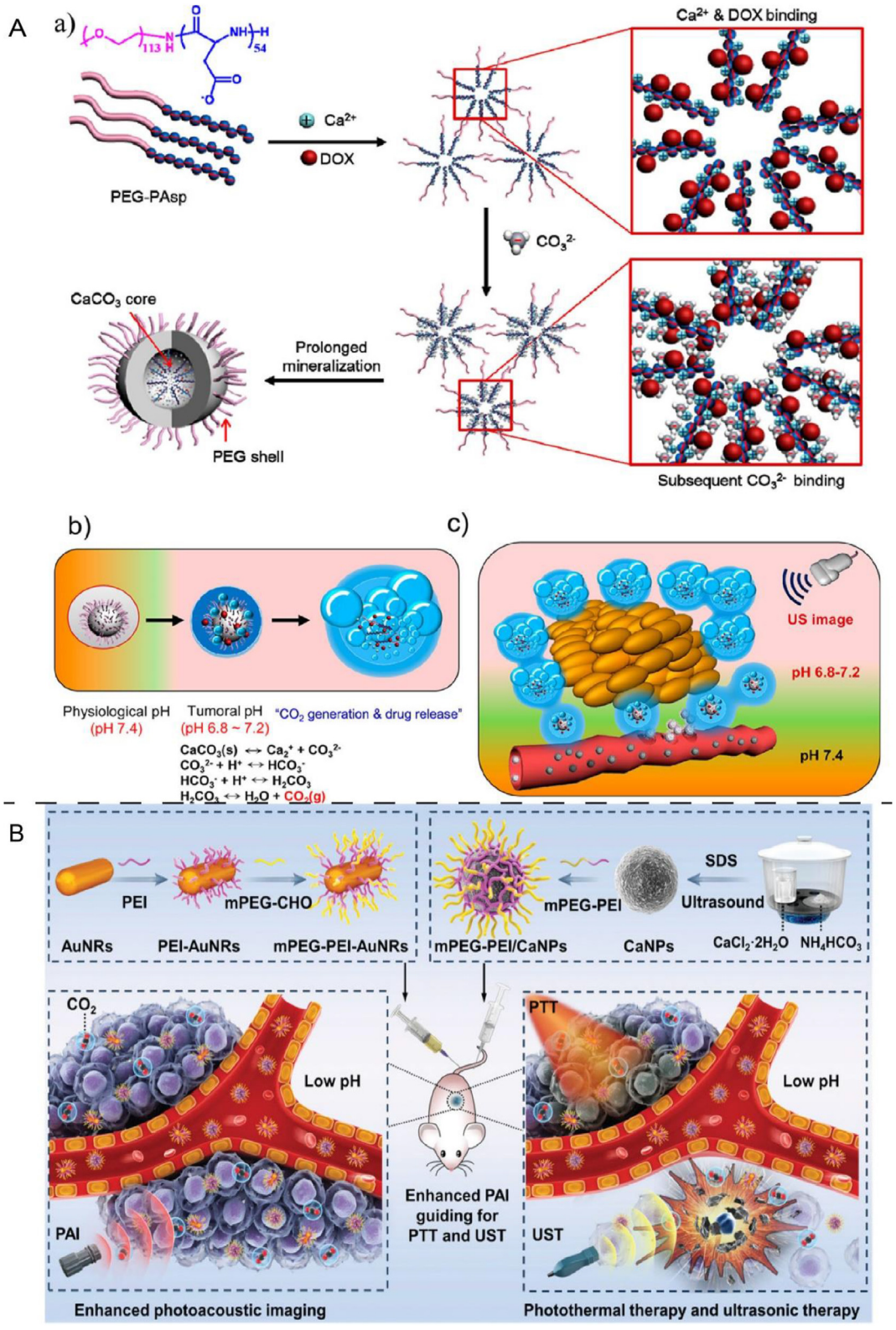

Figure 7 (A) Schematic illustration of (a) fabrication process of DOX-$CaCO_3$-MNPs, MNPs: (b) mechanism of CO_2 generation and drug release, and (c) bubble generation and drug release after accumulation of DOX-$CaCO_3$-MNPs at tumor tissues. MNPs: hybrid nanoparticles. Reprinted with the permission from Ref. 172. Copyright © 2015, American Chemical Society. (B) Schematic illustration showing CO_2 enhanced imaging combining PTT and UST methods. PTT: Photothermal Therapy; UST: ultrasonic Therapy. Reprinted with the permission from Ref. 64. Copyright © 2020 Wiley-VCH GmbH.

which combines the benefits of $CaCO_3$ and nanostructure, has become an attractive avenue for the restoration of bone tissue[183,184].

Recently, researchers synthesized ACC doped with pyrophosphates as stabilizers through an aqueous solution precipitation method, providing new prospects for designing bioactive bone-replacement materials with variable properties[185]. In mice with cranial damage, the use of polyphosphate-implanted ACC ameliorated the proliferation rate of human bone marrow stem cells and yielded a more effective repair effect than standalone components[179]. Poly(lactic-*co*-glycolic acid) microspheres encapsulating ACC doped with a small amount of vaterite demonstrated good biocompatibility *in vivo* and promoted the regeneration of damaged cells at the implanted site[183] (Fig. 8A). Tolba et al.[186] reported the design of ACC encapsulated in a polyphosphate (polyP) scaffold that can stabilize amorphous-phase ACC. Alkaline phosphatase (ALP) is a phosphatase present on bacterial surfaces[187,188]. Following hydrolysis of the polyP framework by ALP, stabilization was abolished, and ACC was readily converted into the final stable crystals along a thermodynamic route. This "delayed carbonization" promotes specific microfracture site healing (Fig. 8B). In addition, calcium-based materials have been found to promote skeletal function repair after surgery for bone tumors. Zhang et al.[189] designed a titanium scaffold loaded with calcium-based nanoparticles, without loading therapeutic drugs, which not only significantly suppressed the solid tumor volume, but also promoted osteogenesis and accelerated bone healing. The findings unequivocally demonstrate the immense potential of ACC for utilization in the domain of tissue engineering, with a particular emphasis on bone repair.

4.6. *Calcium supplement*

Calcium supplements are widely recognized to be beneficial for the bone health of children, young people, and women[190]. Compared to the commonly used crystalline $CaCO_3$, ACC has

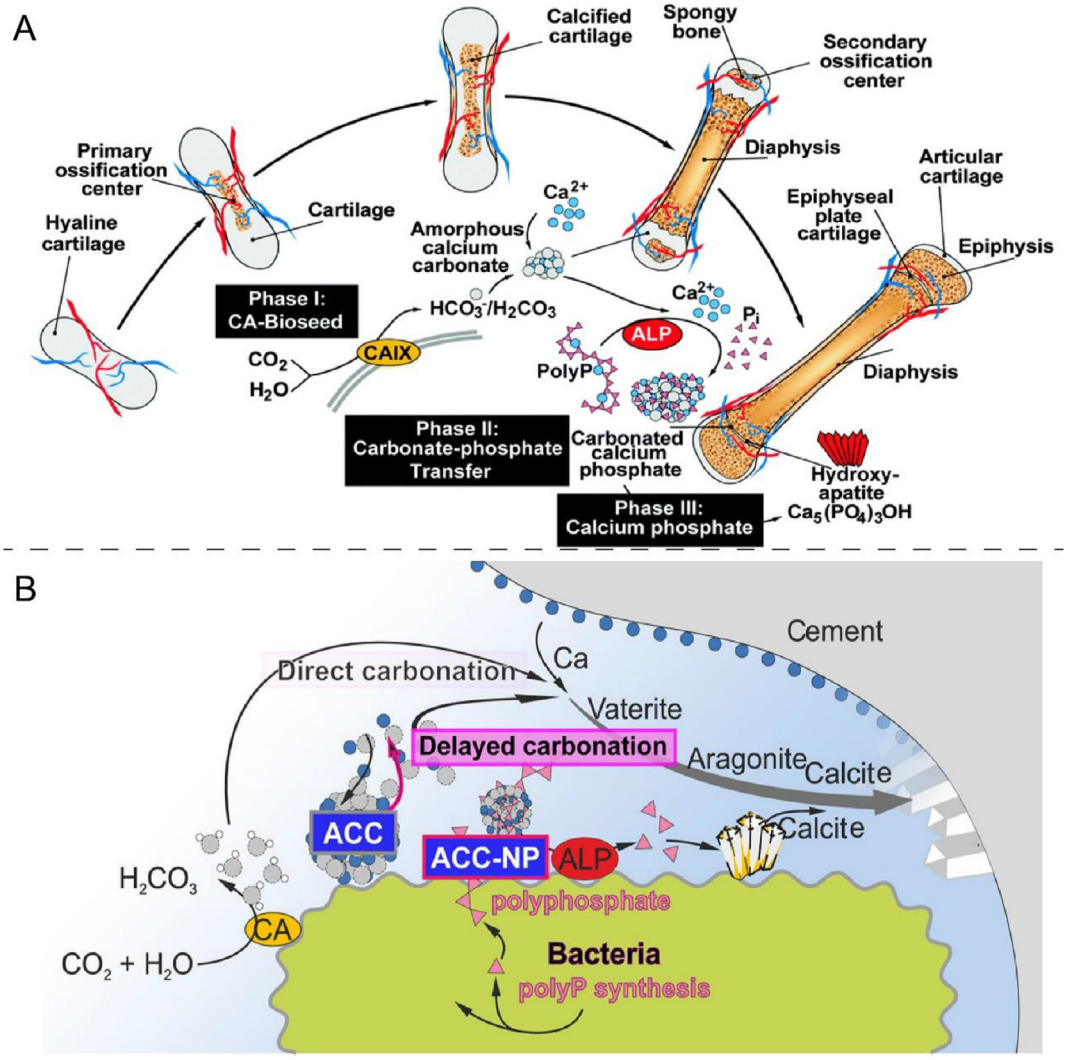

Figure 8 (A) Schematic presentation of the process of endochondral ossification and the proposed phases of bone mineral deposition. CA: carbonic anhydrase. Reprinted with the permission from Ref. 183. Copyright © 2016 Royal Society of Chemistry. (B) Scheme showing the role of the polyP-stabilized ACC nanoparticles, "ACC-NP" in delayed carbonation of cement. Reprinted with the permission from Ref. 139. Copyright © 2020 The Authors. Biotechnology Journal published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

higher solubility and bioavailability, making it a highly effective and readily available dietary calcium source. ACC is approximately 40% more bioavailable than crystalline $CaCO_3$ as a calcium supplement or antacid. Clinical trials have demonstrated that stable ACC has a high absorption rate, which may be a more effective calcium supplement[191]. In addition, experiments comparing natural ACC (crayfish-derived gastrolith powder) and synthetic ACC with two commercially available calcium supplements ($CaCO_3$ and calcium citrate) revealed the significant potential of ACC for the treatment of bone loss by preventing metabolic bone disease and possible osteoporosis[31,190].

Osteoporosis is a common progressive bone disease characterized by reduced bone density and disturbance of bone homeostasis. However, existing therapeutic drugs face problems, such as high doses, low bioavailability, and toxic side effects[192,193]. Promoting osteoblast regeneration is essential for treating osteoporosis, and calcium supplementation is one of the used treatments[194]. To improve the tissue targeting of water-soluble calcium supplements, Wang et al.[195] constructed an ACC-based tetracycline (Tc)-modified and simvastatin (Sim)-loaded phospholipid-ACC hybrid nanoparticle for osteoporotic site-targeted delivery (Tc/ACC/Sim). After intravenous injection, it exerts a dual synergistic effect of *in situ* calcium supplementation and targeted delivery of Sim, a signaling agonist that promotes osteogenesis. The synergistic promotion of the osteoblasts by the combination of calcium supplementation and Sim was superior to nontargeted systems or monotherapy for the treatment of osteoporosis (Fig. 9A). Interestingly, Tao et al.[109] used the water/pH responsiveness of ACC to design an osteoporotic microenvironment-responsive Tc modified and monostearin coated ACC (TMA)/Sim nano platform for oral administration. TMA achieved accumulation of solid calcium supplements at the osteoporosis site and rapid degradation in the osteoporosis microenvironment to efficiently complete the liquid conversion (Fig. 9B). Based on its calcium content and physical properties, ACC is expected to be an excellent calcium supplement.

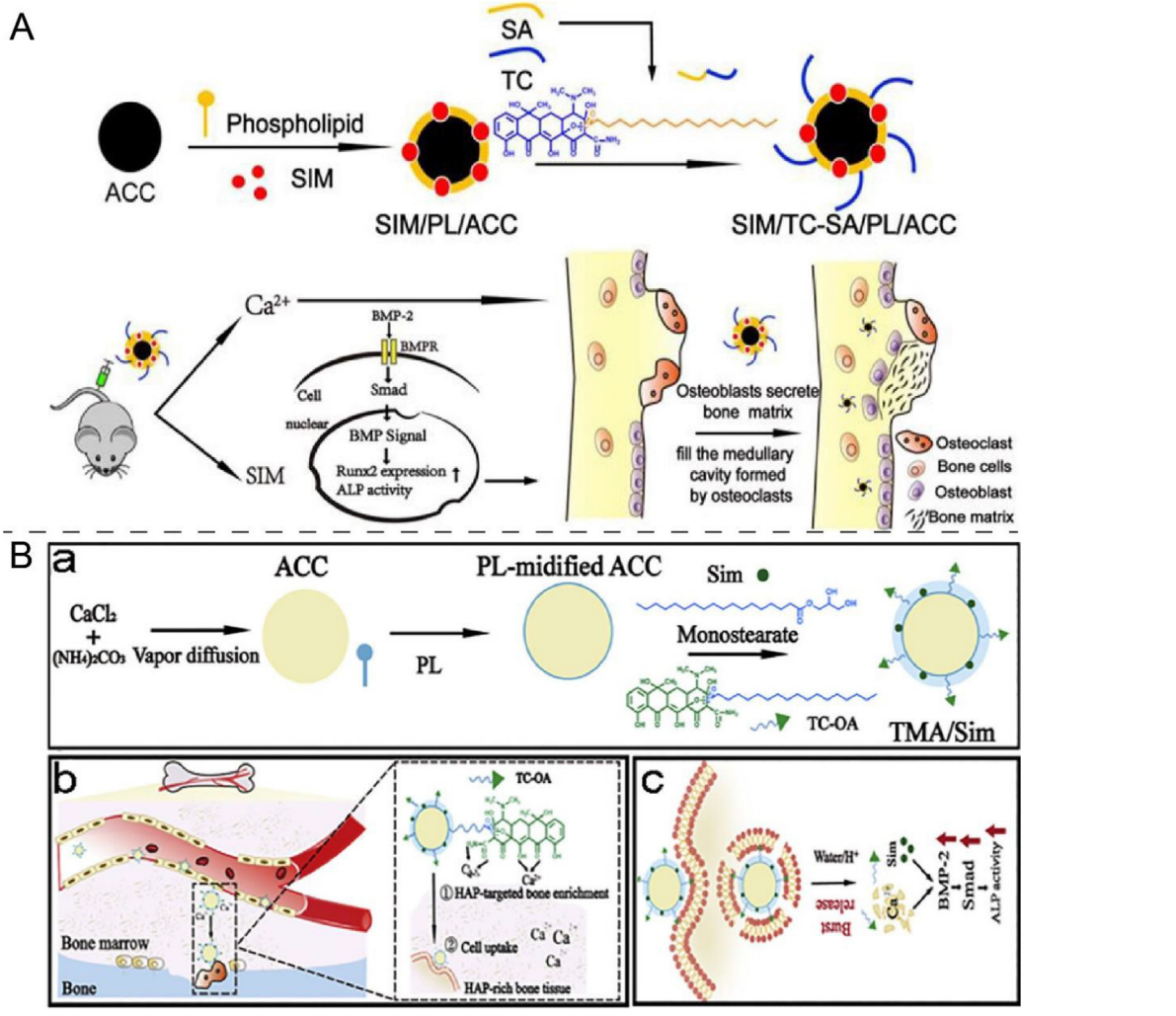

Figure 9 (A) Schematic illustration showing synergistic calcium supplementation and SIM delivery strategy for osteoporosis. SA: stearic acid, BMPs: bone morphogenetic proteins, BMPs-Smad: an important regulatory signaling pathway responsible for the formation of bone. Reprinted with the permission from Ref. 195. Copyright © The authors. (B) (a) Schematic illustration of the construction of TMA/Sim. (b) Schematic illustration of the mechanisms responsible for the targeting and delivery of TMA to target cells in bone. (c) Schematic illustration of the effective osteoporosis reversion effect of the therapeutic regimen using TMA/Sim. Tc-OA: tetracycline-octadecanoic acid conjugate. PL: phospholipid. Reprinted with the permission from Ref. 109. Copyright © 2020 Elsevier B.V.

4.7. *Gene delivery and others*

As previously delineated within the antitumor drug delivery section, the co-precipitation of nano-$CaCO_3$ and DNA represents a promising approach for overcoming multidrug resistance at the genetic level[151]. In addition to DNA delivery alone, synergistic delivery of genes and drugs can also be designed to have higher cytostatic rates than gene or drug delivery alone[196] (Fig. 10A). ACC can also be harnessed for the administration of siRNA, thereby demonstrating good permeability and safety, while also safeguarding siRNA from degradation in tumor tissue[149].

The use of ACC has been identified as a potential strategy for mitigating performance loss and reducing soreness in high-endurance-trained athletes, due to its inherent response to acids[197]. PolyP-stabilized ACC also exhibits the ability to promote the healing of skin lesions[198] (Fig. 10B). Furthermore, ACC-based nano drug delivery systems have antiaging applications. Lactose-modified ACC nanoparticles can target senescent cells with high β-galactosidase activity to exert therapeutic anti-aging effects[199] (Fig. 10C). In addition, $CaCO_3$-nanostructured materials are promising candidates for achieving pH-controlled protein adsorption and release[38]. ATP-stabilized ACC exhibits good hemoglobin adsorption properties[86]. The interaction of ACC with proteins to form a stable amorphous phase provides insights into the development of peptide formulations[200].

ACC can be used to deliver various drugs based on their physical and chemical properties, as shown in Table 2. ACC features inherent nanoscale advantages, such as a porous structure for improved drug loading, pH sensitivity, and water instability, to promote effective drug release at the targeted site. In addition, ACC modified with stabilizers to encapsulate the drug can effectively prevent drug leakage and blood clearance, maintain the stability of the drug system, and improve its bioavailability.

5. Challenges

Nano drug delivery systems based on ACC face several challenges in transporting drugs to specific sites, including macrophage uptake, vascular clearance, and intratumoral pressure, which constrain the effective accumulation of nanoparticles and active and passive targeting mechanisms[130]. Although the addition of stabilizers contributes to the stability of ACC, it may strongly interact with $CaCO_3$, making the system more complex[94]. Meanwhile, inadequate surface modification of ACC can result in drug leakage as it initiates the dissolution of ACC once the delivery system enters the blood[46]. Considering these limitations, it is imperative to develop delivery strategies that reduce drug leakage. At the tumor site, high blood viscosity and pressure may hinder the movement of nanoparticles and reduce permeability. Moreover, it should not be overlooked that there is clearance of nanoparticles by nontargeted tissues with a high flow rate[201]. Therefore, it is essential to design ACC nanoparticles that can effectively penetrate tumors. ACC has been used as a template to synthesize hollow nanostructures. However, significant challenges remain for the development and controlled synthesis of novel hollow nanocarriers. Intensive experiments are required to develop a simple, versatile, and low-cost method for preparing hollow nanoparticles with different morphologies, tunable sizes, and controllable structures[110]. Controlling the batch-to-batch consistency of the nanoparticles is also a critical issue for inorganic materials[13].

Although ACC is considered nontoxic and biodegradable, its long-term toxicity in animals must be investigated before its clinical application[202]. How can the practical application of ACC nanostructured biomaterials in drug delivery be promoted beyond the experimental scale reported in the literature[1]? And do the formulations satisfy the criteria of complete elimination from the

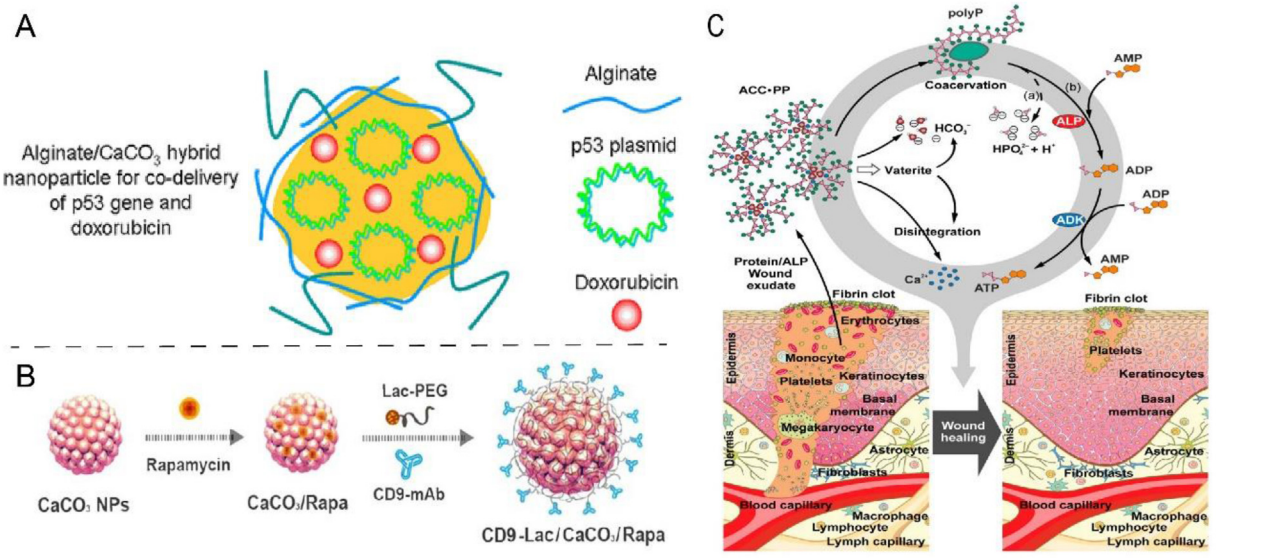

Figure 10 (A) Schematic illustration showing Alginate/$CaCO_3$ hybrid nanoparticles for efficient codelivery of antitumor gene and drug. Reprinted with permission from Ref. 196. Copyright © 2012, American Chemical Society. (B) Schematic representation for CD9 monoclonal antibody-conjugated lactose-wrapped calcium carbonate nanoparticles loaded with rapamycin. Lac: lactose, mAb: monoclonal antibody, Rapa: rapamycin. Reprinted with the permission from Ref. 199. Copyright © 2017 The Authors. (C) Sketch showing the proposed mode of action of "ACC·PP" particles, leading to an acceleration of the wound healing process. ACC·PP: polyP-stabilized ACC. Reprinted with the permission from Ref. 198. Copyright © 2023 by the authors.

Table 2 Biomedical applications of ACC.

Application	Form	Mechanism	Ref.
Self-sacrificing templates	Hollow carbonated hydroxyapatite microspheres with mesoporous structure	A sacrificing Template for hollow nanoparticle therapeutic platform	121
	TCPP-Fe@$CaCO_3$, loaded with L-buthionine sulfoximine		122
	Ultra-small hollow silica nanoparticles		116,120,127
Targeted delivery of antitumor drugs	"Pandora's Box": ACC nanoparticles coated with DOX and monostearic acid	Lipase-triggered water-responsive drug release in lipase-overexpressed tumor tissue	105
	Locking/unlocking strategy: ACC locked with an OA shell and modified with a PEG corona	High aqueous instability of ACC causing burst drug release	62
	ACC stabilized by poly(acrylic acid) and loaded with DOX	The pH-responsive property facilitating targeted drug release	96
	Phosphate-stabilized ACC/sodium alginate composite NPs	ACC decomposes with the presence of H^+ while sodium alginate regulates the release rate, resulting in a responsive and sustained-release effect	150
	A novel nanoreactor of ACC−DOX@silica	1. DOX is encapsulated in the form of Ca-DOX complexes DOX 2. Negatively charged silanol groups interact electrostatically with released DOX under acidic conditions, achieving a sustained-release effect	28
	Mn^{2+}-doped ACC nanoparticles, loaded with ICG and IMQ	ACC nanoparticles remove tumor-derived H^+, allowing simultaneous delivery of ICG and IMQ into the tumor	146
	Fluorination ACC-DOX silica NPs modified with a PL layer	pH responsiveness & Cavitation activities triggered by the US	152
Regulation of immune function	Honeycomb $CaCO_3$ with ovalbumin as a skeleton	1. Reduce the H^+ in the tumor microenvironment 2. Generate Ca^{2+} to disrupt the autophagy inhibition DCs 3. Mediate the release of DAMPs from tumor cells to promote the maturation of DCs	162
	TCPP-Fe@$CaCO_3$ loaded with L-buthionine sulfoximine	Rapid release of Ca^{2+} and L-buthionine sulfoximine in an acidic tumor microenvironment leads to Ca^{2+} overload-induced mitochondrial damage and amplification of tumor oxidative stress	122
Enhancement of tumor imaging	DOX-loaded $CaCO_3$ hybrid nanoparticles	Drug release and generation of CO_2 required for US imaging are achieved under acidic conditions	172
	$CaCO_3$ modified by PEG and polyethyleneimine	Production of CO_2 bubbles leads to enhanced photoacoustic signals	64
	Ultrafine paramagnetic amorphous carbonate nanoclusters with gadolinium ions and poly(acrylic acid)	Mutual effects between the paramagnetic lanthanide gadolinium ion and ACC contributes to the transparent Magnetic Resonance Imaging contrasting enhancement of gadolinium-based nanoclusters	173
	A dual-mode imaging (US and fluorescence) diagnostic nanoparticle system: $CaCO_3$ loaded with DOX and indocyanine green	1. ACC undergoes decomposition exposed to an acidic environment, facilitating the release of DOX and generating CO_2 to enhance US imaging 2. ICG is employed for further fluorescent detection of the tumor	169
Tissue engineering	Pyrophosphate-stabilized ACC.	ACC exhibits excellent biocompatibility and the ability to release ions that compose bone minerals	185
	Combinations of polyP and ACC microparticles	1. Combinations act as bioseeds for the deposition of bone minerals and as inducers of CAIX gene expression 2. They induce ALP, promoting the release of inorganic phosphate	179
	PolyP stabilized ACC	The system induced the expression of ALP gene	183,186
Calcium supplement	ACC capsules containing 192 mg of elemental calcium	A higher bioavailability of calcium from ACC compared to crystalline $CaCO_3$	191
	A Tc modified and Sim-loaded PL- ACC hybrid NPs	Calcium supplements, in combination with Sim, activate BMP-2 in osteoblasts through the BMP-Smad signaling pathway, thereby promoting the formation of new bone	195
	A Tc modified and MS-coated ACC platform loaded with Sim		109

Table 2 (*continued*)

Application	Form	Mechanism	Ref.
Gene Delivery	ACC/Ca(II)-IP6 compound NPs- siRNA complexes	Non-viral gene carriers efficiently transfer siRNA to target cells with high transfection efficiency, ensuring the protection of siRNA from degradation	149
	Alginate/$CaCO_3$ nanoparticles with DOX and P53 expression plasmid	Ca^{2+} forms a co-precipitate with DNA, facilitating the synergistic delivery of genes and drugs	196
Pain Relief	ACC supplementation (2000 mg per day)	ACC exhibits enhanced buffering capacity, potentially providing faster and more effective H^+ buffering	197
Wound Healing	PolyP-stabilized ACC	The migration propensity of endothelial cells is increased	198

TCPP, meso-tetra-(4-carboxyphenyl) porphine; ACC, amorphous calcium carbonate; DOX, doxorubicin; OA, oleic acid; PEG, polyethylene glycol; NPs, nanoparticles; ICG, indocyanine green; IMQ, imiquimod; PL, phospholipid; US, ultrasound; polyp, polyphosphate; CA, carbonic anhydrase; ALP, alkaline phosphatase; Tc, tetracycline; Sim, simvastatin; BMPs, bone morphogenetic proteins.

body, complete non-toxicity, and biodegradability[136]? These are all issues that must be considered. Simultaneously, the materials of a carrier that has not received approval from the medical committees could result in the ineffectiveness of clinical translation[25]. Consequently, a methodical examination of the prospective enduring safety, toxicological implications, biodegradability, biocompatibility, and availability of ACC is necessary[181].

6. Future

Nanosized ACC presents a promising future with the advantages of being safe, non-toxic, biodegradable, responsive, abundant, and has a wide range of applications[203,204]. Unlike other inorganic nanomaterials, ACC can be decomposed *in vivo* to generate Ca^{2+}, which is involved in the regulation of physiological and pathological processes in living organisms[13]. The synthesis method of ACC has the advantages of simplicity and convenience and tends to be environmentally friendly. ACC displays higher reactivity than crystalline $CaCO_3$, although it is unstable under normal aqueous atmospheric conditions. Based on this point, we can take full advantage of its environmental responsiveness to design simple and feasible strategies for the optimization of ACC to suit industrial production[205]. Regardless of tumor-targeted delivery, bone regeneration promotion, immune modulation, and enhanced imaging, it is strongly suggested that ACC nanoparticles be functionally modified to achieve improved therapeutic effects.

With multifunctional intelligent materials gaining popularity in drug delivery, biosafety will remain a critical criterion for assessment. For ultimate clinical application, factors, including stability, homogeneity, and the feasibility of mass production, must also be considered. As a delivery carrier with multiple advantages, ACC necessitates intense basic and clinical research to promote and support its use in clinical treatments.

7. Conclusions

Nanometer-sized ACC, as a biocompatible and biodegradable inorganic nanoparticle, overcomes the inherent limitations of micrometer-sized crystalline $CaCO_3$. Its high solubility allows for better absorption, resulting in increased absorbability and higher bioavailability. Moreover, functional ACC exhibits excellent transfection efficiency, lower cytotoxicity, and osteogenic potential, making them promising alternatives for gene delivery vectors and tissue engineering materials. ACC has a wide range of sources and a simple synthesis pathway, but further research strategies and modifications are needed to control its stability. In summary, ACC has many advantages as a drug carrier, but the barrier of water instability must be overcome. Importantly, the clinical application of ACC remains limited. Long-term studies are crucial for assessing the effectiveness, persistence, and long-term safety of ACC. Additionally, more research and understanding are needed to precisely control the properties of ACC to meet the diverse clinical requirements. Therefore, to drive the clinical application of ACC forward, consistent attention is required.

Acknowledgments

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Author contributions

Han Liu: Conceptualization and Writing – original draft. Zhiyang Wen, Zihan Liu, Hongliang Wang, Yanfang Yang, and Xuejun Xia: Writing – review & editing. Jun Ye and Yuling Liu: Conceptualization, Editing, Supervision, Funding acquisition.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Yi LJ, Li JF, Ma MG, Zhu YJ. Nanostructured calcium-based biomaterials and their application in drug delivery. *Curr Med Chem* 2020;**27**:5189–212.
2. Chow EK-H, Ho D. Cancer Nanomedicine: from drug delivery to imaging. *Sci Transl Med* 2013;**5**:216rv4.
3. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. *ACS Nano* 2009;**3**:16–20.

4. Nicolas J, Mura S, Brambilla D, Mackiewicz N, Couvreur P. Design, functionalization strategies and biomedical applications of targeted biodegradable/biocompatible polymer-based nanocarriers for drug delivery. *Chem Soc Rev* 2013;**42**:1147−235.
5. Harish V, Tewari D, Gaur M, Yadav AB, Swaroop S, Bechelany M, et al. Review on nanoparticles and nanostructured materials: bioimaging, biosensing, drug delivery, tissue engineering, antimicrobial, and agro-food applications. *Nanomaterials (Basel)* 2022;**12**:457.
6. Sahoo SK, Labhasetwar V. Nanotech approaches to drug delivery and imaging. *Drug Discov Today* 2003;**8**:1112−20.
7. Shi J, Votruba AR, Farokhzad OC, Langer R. Nanotechnology in drug delivery and Tissue Engineering: from discovery to applications. *Nano Lett* 2010;**10**:3223−30.
8. Liong M, Lu J, Kovochich M, Xia T, Ruehm SG, Nel AE, et al. Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *ACS Nano* 2008;**2**:889−96.
9. Naz S, Shamoon M, Wang R, Zhang L, Zhou J, Chen J. Advances in therapeutic implications of inorganic drug delivery nano-platforms for cancer. *Int J Mol Sci* 2019;**20**:965.
10. Choi HS, Liu W, Liu F, Nasr K, Misra P, Bawendi MG, et al. Design considerations for tumour-targeted nanoparticles. *Nat Nanotechnol* 2010;**5**:42−7.
11. Xu A-W, Yu Q, Dong W-F, Antonietti M, Cölfen H. Stable Amorphous $CaCO_3$ microparticles with hollow spherical superstructures stabilized by phytic acid. *Adv Mater (Weinheim, Ger)* 2005;**17**:2217−21.
12. Wei W, Ma G-H, Hu G, Yu D, McLeish T, Su ZG, et al. Preparation of hierarchical hollow $CaCO_3$ particles and the application as an anticancer drug carrier. *J Am Chem Soc* 2008;**130**:15808−10.
13. Qi C, Lin J, Fu LH, Huang P. Calcium-based biomaterials for diagnosis, treatment, and theranostics. *Chem Soc Rev* 2018;**47**:357−403.
14. Zamponi GW. Targeting voltage-gated calcium channels in neurological and psychiatric diseases. *Nat Rev Drug Discov* 2016;**15**:19−34.
15. Chumnarnsilpa S, Robinson RC, Grimes JM, Leyrat C. Calcium-controlled conformational choreography in the N-terminal half of adseverin. *Nat Commun* 2015;**6**:8254.
16. Naka K, Chujo Y. Control of Crystal nucleation and growth of calcium carbonate by synthetic substrates. *Chem Mater* 2001;**13**:3245−59.
17. Trushina DB, Bukreeva TV, Kovalchuk MV, Antipina MN. $CaCO_3$ vaterite microparticles for biomedical and personal care applications. *Mater Sci Eng C* 2014;**45**:644−58.
18. Cartwright JH, Checa AG, Gale JD, Gebauer D, Sainz-Díaz CI. Calcium carbonate polyamorphism and its role in biomineralization: how many amorphous calcium carbonates are there?. *Angew Chem Int Ed Engl* 2012;**51**:11960−70.
19. Tuukkanen J, Nakamura M. Hydroxyapatite as a nanomaterial for advanced tissue engineering and drug therapy. *Curr Pharmaceut Des* 2017;**23**:3786−93.
20. Ueno Y, Futagawa H, Takagi Y, Ueno A, Mizushima Y. Drug-incorporating calcium carbonate nanoparticles for a new delivery system. *J Control Release* 2005;**103**:93−8.
21. Dong Z, Feng L, Hao Y, Chen M, Gao M, Chao Y, et al. Synthesis of hollow biomineralized $CaCO_3$−polydopamine nanoparticles for multimodal imaging-guided cancer photodynamic therapy with reduced skin photosensitivity. *J Am Chem Soc* 2018;**140**:2165−78.
22. Fu J, Leo CP, Show PL. Recent advances in the synthesis and applications of pH-responsive $CaCO_3$. *Biochem Eng J* 2022;**187**:108446.
23. Nebel H, Neumann M, Mayer C, Epple M. On the structure of amorphous calcium carbonate—a detailed study by solid-state NMR spectroscopy. *Inorg Chem* 2008;**47**:7874−9.
24. Sun R, Zhang P, Bajnoczi EG, Neagu A, Tai CW, Persson I, et al. Amorphous calcium carbonate constructed from nanoparticle aggregates with unprecedented surface area and mesoporosity. *ACS Appl Mater Interfaces* 2018;**10**:21556−64.
25. Trofimov AD, Ivanova AA, Zyuzin MV, Timin AS. Porous inorganic carriers based on silica, calcium carbonate and calcium phosphate for controlled/modulated drug delivery: fresh outlook and future perspectives. *Pharmaceutics* 2018;**10**:167.
26. Trushina DB, Borodina TN, Belyakov S, Antipina MN. Calcium carbonate vaterite particles for drug delivery: advances and challenges. *Mater Today Adv* 2022;**14**:100214.
27. Niu YQ, Liu JH, Aymonier C, Fermani S, Kralj D, Falini G, et al. Calcium carbonate: controlled synthesis, surface functionalization, and nanostructured materials. *Chem Soc Rev* 2022;**51**:7883−943.
28. Zhao Y, Luo Z, Li M, Qu Q, Ma X, Yu SH, et al. A preloaded amorphous calcium carbonate/doxorubicin@silica nanoreactor for pH-responsive delivery of an anticancer drug. *Angew Chem Int Ed Engl* 2015;**54**:919−22.
29. Addadi L, Raz S, Weiner S. Taking advantage of disorder: amorphous calcium carbonate and its roles in biomineralization. *Adv Mater (Weinheim, Ger)* 2003;**15**:959−70.
30. Prenant M. Les formes minÉralogiques du calcaire chez les Êtres vivants, et Le problÈme de leur dÉterminisme. *Biol Rev* 1927;**2**:365−93.
31. Shaltiel G, Bar-David E, Meiron OE, Waltman E, Shechter A, Aflalo ED, et al. Bone loss prevention in ovariectomized rats using stable amorphous calcium carbonate. *Health* 2013;**5**:18−29.
32. Foran E, Weiner S, Fine M. Biogenic fish-gut calcium carbonate is a stable amorphous phase in the gilt-head seabream, sparus aurata. *Sci Rep* 2013;**3**:1700.
33. Neira-Carrillo A, Fernández MS, Hevia GP, Arias JL, Gebauer D, Cölfen H. Retrosynthesis of $CaCO_3$ *via* amorphous precursor particles using gastroliths of the red claw lobster (cherax quadricarinatus). *J Struct Biol* 2017;**199**:46−56.
34. Weiner S, Sagi I, Addadi L. Structural biology. Choosing the crystallization path less traveled. *Science* 2005;**309**:1027−8.
35. Mass T, Giuffre AJ, Sun CY, Stifler CA, Frazier MJ, Neder M, et al. Amorphous calcium carbonate particles form coral skeletons. *PANS (Pest Artic News Summ)* 2017;**114**:E7670−8.
36. Habraken WJ, Masic A, Bertinetti L, Al-Sawalmih A, Glazer L, Bentov S, et al. Layered growth of crayfish gastrolith: about the stability of amorphous calcium carbonate and role of additives. *J Struct Biol* 2015;**189**:28−36.
37. Hodson ME, Benning LG, Demarchi B, Penkman KE, Rodriguez-Blanco JD, Schofield PF, et al. Biomineralisation by earthworms—an investigation into the stability and distribution of amorphous calcium carbonate. *Geochem Trans* 2015;**16**:4.
38. Qi C, Zhu YJ, Chen F. Microwave hydrothermal transformation of amorphous calcium carbonate nanospheres and application in protein adsorption. *ACS Appl Mater Interfaces* 2014;**6**:4310−20.
39. Zou Z, Yang X, Albéric M, Heil T, Wang Q, Pokroy B, et al. Additives control the stability of amorphous calcium carbonate *via* two different mechanisms: surface adsorption versus bulk incorporation. *Adv Funct Mater* 2020;**30**:2000003.
40. Sun R, Tai CW, Strømme M, Cheung O. The effects of additives on the porosity and stability of amorphous calcium carbonate. *Microporous Mesoporous Mater* 2020;**292**:109736.
41. Tobler DJ, Rodriguez-Blanco JD, Dideriksen K, Bovet N, Sand KK, Stipp SLS. Citrate effects on amorphous calcium carbonate (ACC) structure, stability, and crystallization. *Adv Funct Mater* 2015;**25**:3081−90.
42. Chen SF, Colfen H, Antonietti M, Yu SH. Ethanol assisted synthesis of pure and stable amorphous calcium carbonate nanoparticles. *Chem Commun (Camb)* 2013;**49**:9564−6.
43. Xue CC, Li MH, Zhao Y, Zhou J, Hu Y, Cai KY, et al. Tumor microenvironment-activatable Fe-doxorubicin preloaded amorphous $CaCO_3$ nanoformulation triggers ferroptosis in target tumor cells. *Sci Adv* 2020;**6**:eaax1346.
44. Xto JM, Borca CN, van Bokhoven JA, Huthwelker T. Aerosol-based synthesis of pure and stable amorphous calcium carbonate. *Chem Commun (Camb)* 2019;**55**:10725−8.

45. Blue CR, Rimstidt JD, Dove PM. A mixed flow reactor method to synthesize amorphous calcium carbonate under controlled chemical conditions. *Methods Enzymol* 2013;**532**:557−68.
46. Konrad F, Gallien F, Gerard DE, Dietzel M. Transformation of amorphous calcium carbonate in air. *Cryst Growth Des* 2016;**16**: 6310−7.
47. Ihli J, Kulak AN, Meldrum FC. Freeze-drying yields stable and pure amorphous calcium carbonate (ACC). *Chem Commun (Cambridge, U K)* 2013;**49**:3134−6.
48. Rao C, Guo X, Li M, Sun X, Lian X, Wang H, et al. *In vitro* preparation and characterization of amorphous calcium carbonate nanoparticles for applications in curcumin delivery. *J Mater Sci* 2019;**54**: 11243−53.
49. Som A, Raliya R, Tian L, Akers W, Ippolito JE, Singamaneni S, et al. Monodispersed calcium carbonate nanoparticles modulate local pH and inhibit tumor growth *in vivo*. *Nanoscale* 2016;**8**:12639−47.
50. Wang C, Liu X, Chen S, Hu F, Sun J, Yuan H. Facile preparation of phospholipid-amorphous calcium carbonate hybrid nanoparticles: toward controllable burst drug release and enhanced tumor penetration. *Chem Commun (Camb)* 2018;**54**:13080−3.
51. Wang C, Han M, Liu X, Chen S, Hu F, Sun J, et al. Mitoxantrone-preloaded water-responsive phospholipid-amorphous calcium carbonate hybrid nanoparticles for targeted and effective cancer therapy. *Int J Nanomed* 2019;**14**:1503−17.
52. Sun S, Gebauer D, Colfen H. A general strategy for colloidal stable ultrasmall amorphous mineral clusters in organic solvents. *Chem Sci* 2017;**8**:1400−5.
53. Gal J, Bollinger J, Tolosa H, Gache N. Calcium carbonate solubility: a reappraisal of scale formation and inhibition. *Talanta* 1996;**43**: 1497−509.
54. Schmidt I, Zolotoyabko E, Lee K, Gjardy A, Berner A, Lakin E, et al. Effect of strontium ions on crystallization of amorphous calcium carbonate. *Cryst Res Technol* 2019;**54**:1900002.
55. Chuzeville L, Boury F, Duday D, Anand R, Moretto E, Thomann JS. Eco-friendly processes for the synthesis of amorphous calcium carbonate nanoparticles in ethanol and their stabilisation in aqueous media. *Green Chem* 2022;**24**:1270−84.
56. Zeng Y, Cao J, Wang Z, Guo J, Lu J. Formation of amorphous calcium carbonate and its transformation mechanism to crystalline $CaCO_3$ in laminar microfluidics. *Cryst Growth Des* 2018;**18**:1710−21.
57. Hu D, Zhang W, Tang J, Zhou Z, Liu X, Shen Y. Improving safety of cancer immunotherapy *via* delivery technology. *Biomaterials* 2021; **265**:120407.
58. Mao X, Wang X, Jin M, Li Q, Jia J, Li M, et al. Critical involvement of lysyl oxidase in seizure-induced neuronal damage through ERK-Alox5-dependent ferroptosis and its therapeutic implications. *Acta Pharm Sin B* 2022;**12**:3513−28.
59. Ju YM, Zhao Y, Guan QF, Yang SY, Wang W, Yan BB, et al. Amorphous calcium carbonate cluster nanospheres in water-deficient organic solvents. *Angew Chem Int Ed* 2022;**61**:e202211254.
60. Clark SM, Colas B, Jacob DE, Neuefeind JC, Wang HW, Page KL, et al. The nano- and meso-scale structure of amorphous calcium carbonate. *Sci Rep* 2022;**12**:6870.
61. Meiron OE, Bar-David E, Aflalo ED, Shechter A, Stepensky D, Berman A, et al. Solubility and bioavailability of stabilized amorphous calcium carbonate. *J Bone Miner Res* 2011;**26**:364−72.
62. Wang C, Chen S, Yu Q, Hu F, Yuan H. Taking advantage of the disadvantage: employing the high aqueous instability of amorphous calcium carbonate to realize burst drug release within cancer cells. *J Mater Chem B* 2017;**5**:2068−73.
63. Pellow C, O'Reilly MA, Hynynen K, Zheng G, Goertz DE. Simultaneous intravital optical and acoustic monitoring of ultrasound-triggered nanobubble generation and extravasation. *Nano Lett* 2020;**20**:4512−9.
64. Xu C, Wang Y, Wang E, Yan N, Sheng S, Chen J, et al. Effective eradication of tumors by enhancing photoacoustic-imaging-guided combined photothermal therapy and ultrasonic therapy. *Adv Funct Mater* 2020;**31**:2009314.
65. Shi X, Bi Y, Yang W, Guo X, Jiang Y, Wan C, et al. Ca^{2+} regulates T-cell receptor activation by modulating the charge property of lipids. *Nature* 2013;**493**:111−5.
66. Monteith GR, Prevarskaya N, Roberts-Thomson SJ. The calcium-cancer signalling nexus. *Nat Rev Cancer* 2017;**17**:367−80.
67. An J, Liu M, Zhao L, Lu W, Wu S, Zhang K, et al. Boosting tumor immunotherapy by bioactive nanoparticles *via* Ca^{2+} interference mediated TME reprogramming and specific PD-L1 depletion. *Adv Funct Mater* 2022;**32**:2201275.
68. Park S-H, Hwang I, McNaughton DA, Kinross AJ, Howe ENW, He Q, et al. Synthetic Na^+/K^+ exchangers promote apoptosis by disturbing cellular cation homeostasis. *Chem* 2021;**7**:3325−39.
69. Loncke J, Kaasik A, Bezprozvanny I, Parys JB, Kerkhofs M, Bultynck G. Balancing er-mitochondrial Ca^{2+} fluxes in health and disease. *Trends Cell Biol* 2021;**31**:598−612.
70. Ma Z, Zhang J, Zhang W, Foda MF, Zhang Y, Ge L, et al. Intracellular Ca^{2+} cascade guided by NIR-II photothermal switch for specific tumor therapy. *iScience* 2020;**23**:101049.
71. Pouget EM, Bomans PHH, Goos JACM, Frederik PM, de With G, Sommerdijk NAJM. The initial stages of template-controlled $CaCO_3$ formation revealed by cryo-TEM. *Science* 2009;**323**:1455−8.
72. Rodriguez-Blanco JD, Shaw S, Benning LG. The kinetics and mechanisms of amorphous calcium carbonate (ACC) crystallization to calcite, *via* vaterite. *Nanoscale* 2011;**3**:265−71.
73. Alberic M, Bertinetti L, Zou Z, Fratzl P, Habraken W, Politi Y. The crystallization of amorphous calcium carbonate is kinetically governed by ion impurities and water. *Adv Sci (Weinh)* 2018;**5**:1701 000.
74. Du H, Courrégelongue C, Xto J, Böhlen A, Steinacher M, Borca CN, et al. Additives: their influence on the humidity- and pressure-induced crystallization of amorphous $CaCO_3$. *Chem Mater* 2020; **32**:4282−91.
75. Bentov S, Weil S, Glazer L, Sagi A, Berman A. Stabilization of amorphous calcium carbonate by phosphate rich organic matrix proteins and by single phosphoamino acids. *J Struct Biol* 2010;**171**: 207−15.
76. Cantaert B, Kuo D, Matsumura S, Nishimura T, Sakamoto T, Kato T. Use of amorphous calcium carbonate for the design of new materials. *ChemPlusChem* 2017;**82**:107−20.
77. Ihli J, Kim YY, Noel EH, Meldrum FC. The effect of additives on amorphous calcium carbonate (ACC): Janus behavior in solution and the solid state. *Adv Funct Mater* 2013;**23**:1575−85.
78. Opitz P, Asta MP, Fernandez-Martinez A, Panthöfer M, Kabelitz A, Emmerling F, et al. Monitoring a mechanochemical reaction reveals the formation of a new ACC defect variant containing the HCO_3^- anion encapsulated by an amorphous matrix. *Cryst Growth Des* 2020; **20**:6831−46.
79. Zhang J, Zhou X, Dong C, Sun Y, Yu J. Investigation of amorphous calcium carbonate's formation under high concentration of magnesium: the prenucleation cluster pathway. *J Cryst Growth* 2018;**494**: 8−16.
80. Konrad F, Purgstaller B, Gallien F, Mavromatis V, Gane P, Dietzel M. Influence of aqueous Mg concentration on the transformation of amorphous calcium carbonate. *J Cryst Growth* 2018;**498**: 381−90.
81. Aizenberg J, Lambert G, Weiner S, Addadi L. Factors involved in the formation of amorphous and crystalline calcium carbonate: a study of an ascidian skeleton. *J Am Chem Soc* 2002;**124**:32−9.
82. Koishi A, Fernandez-Martinez A, Ruta B, Jimenez-Ruiz M, Poloni R, di Tommaso D, et al. Role of impurities in the kinetic persistence of amorphous calcium carbonate: a nanoscopic dynamics view. *J Phys Chem C* 2018;**122**:16983−91.
83. Reddy MM, Nancollas GH. The crystallization of calcium carbonate. *J Cryst Growth* 1976;**35**:33−8.
84. Blue CR, Giuffre A, Mergelsberg S, Han N, De Yoreo JJ, Dove PM. Chemical and physical controls on the transformation of amorphous calcium carbonate into crystalline $CaCO_3$ polymorphs. *Geochem Cosmochim Acta* 2017;**196**:179−96.

85. Gal A, Weiner S, Addadi L. The stabilizing effect of silicate on biogenic and synthetic amorphous calcium carbonate. *J Am Chem Soc* 2010;**132**:13208−11.
86. Qi C, Zhu YJ, Lu BQ, Zhao XY, Zhao J, Chen F, et al. ATP-stabilized amorphous calcium carbonate nanospheres and their application in protein adsorption. *Small* 2014;**10**:2047−56.
87. Cheang TY, Wang SM, Hu ZJ, Xing ZH, Chang GQ, Yao C, et al. Calcium carbonate/CaIP6 nanocomposite particles as gene delivery vehicles for human vascular smooth muscle cells. *J Mater Chem* 2010;**20**:8050−5.
88. Cai A, Xu X, Pan H, Tao J, Liu R, Tang R, et al. Direct synthesis of hollow vaterite nanospheres from amorphous calcium carbonate nanoparticles *via* phase transformation. *J Phys Chem C* 2008;**112**: 11324−30.
89. Zou Z, Bertinetti L, Politi Y, Fratzl P, Habraken W. Control of polymorph selection in amorphous calcium carbonate crystallization by poly(aspartic acid): two different mechanisms. *Small* 2017;**13**: 1603100.
90. Huang W, Wang Q, Chi W, Cai M, Wang R, Fu Z, et al. Multiple crystallization pathways of amorphous calcium carbonate in the presence of poly(aspartic acid) with a chain length of 30. *CrystEngComm* 2022;**24**:4809−18.
91. Xu X, Liu H, Guo J, Huo Z, Liu J, Wu Z, et al. Intragastric amorphous calcium carbonate consumption triggered generation of *in situ* hydrogel piece for sustained drug release. *Int J Pharm* 2020;**590**: 119880.
92. Bassett DC, Marelli B, Nazhat SN, Barralet JE. Stabilization of amorphous calcium carbonate with nanofibrillar biopolymers. *Adv Funct Mater* 2012;**22**:3460−9.
93. Mergelsberg ST, De Yoreo JJ, Miller QRS, Marc Michel F, Ulrich RN, Dove PM. Metastable solubility and local structure of amorphous calcium carbonate (ACC). *Geochem Cosmochim Acta* 2020;**289**:196−206.
94. Kellermeier M, Gebauer D, Melero-García E, Drechsler M, Talmon Y, Kienle L, et al. Colloidal stabilization of calcium carbonate prenucleation clusters with silica. *Adv Funct Mater* 2012;**22**: 4301−11.
95. Mejri W, Korchef A, Tlili M, Ben Amor M. Effects of temperature on precipitation kinetics and microstructure of calcium carbonate in the presence of magnesium and sulphate ions. *Desalin Water Treat* 2013; **52**:4863−70.
96. Xu C, Yan Y, Tan J, Yang D, Jia X, Wang L, et al. Biodegradable nanoparticles of polyacrylic acid-stabilized amorphous $CaCO_3$ for tunable pH-responsive drug delivery and enhanced tumor inhibition. *Adv Funct Mater* 2019;**29**:1801846.
97. Lima PHCd, Butera AP, Cabeça LF, Ribeiro-Viana RM. Liposome surface modification by phospholipid chemical reactions. *Chem Phys Lipids* 2021;**237**:105084.
98. Needham D, McIntosh TJ, Lasic DD. Repulsive interactions and mechanical stability of polymer-grafted lipid membranes. *Biochim Biophys Acta Biomembr* 1992;**1108**:40−8.
99. Berendsen HJC, Marrink SJ. Molecular dynamics of water transport through membranes: water from solvent to solute. *Pure Appl Chem* 1993;**65**:2513−20.
100. Marrink SJ, Berendsen HJC. Simulation of water transport through a lipid membrane. *J Phys Chem C* 1994;**98**:4155−68.
101. Orsi M, Sanderson WE, Essex JW. Permeability of small molecules through a lipid bilayer: a multiscale simulation study. *J Phys Chem B* 2009;**113**:12019−29.
102. Abu Lila AS, Kiwada H, Ishida T. The accelerated blood clearance (ABC) phenomenon: clinical challenge and approaches to manage. *J Control Release* 2013;**172**:38−47.
103. Torchilin VP. Polymer-coated long-circulating microparticulate pharmaceuticals. *J Microencapsul* 1998;**15**:1−19.
104. Lasic DD, Martin FJ, Gabizon A, Huang SK, Papahadjopoulos D. Sterically stabilized liposomes: a hypothesis on the molecular origin of the extended circulation times. *Biochim Biophys Acta* 1991;**1070**: 187−92.
105. Wang C, Chen S, Wang Y, Liu X, Hu F, Sun J, et al. Lipase-triggered water-responsive "pandora's box" for cancer therapy: toward induced neighboring effect and enhanced drug penetration. *Adv Mater* 2018; **30**:e1706407.
106. Kellermeier M, Melero-García E, Glaab F, Klein R, Drechsler M, Rachel R, et al. Stabilization of amorphous calcium carbonate in inorganic silica-rich environments. *J Am Chem Soc* 2010;**132**: 17859−66.
107. Yohe ST, Colson YL, Grinstaff MW. Superhydrophobic materials for tunable drug release: using displacement of air to control delivery rates. *J Am Chem Soc* 2012;**134**:2016−9.
108. Chabner BA, Roberts TG. Chemotherapy and the war on cancer. *Nat Rev Cancer* 2005;**5**:65−72.
109. Tao S, Yu F, Song Y, Zhou W, Lv J, Zhao R, et al. Water/pH dual responsive *in situ* calcium supplement collaborates simvastatin for osteoblast promotion mediated osteoporosis therapy *via* oral medication. *J Control Release* 2021;**329**:121−35.
110. Teng Z, Li W, Tang Y, Elzatahry A, Lu G, Zhao D. Mesoporous organosilica hollow nanoparticles: synthesis and applications. *Adv Mater* 2019;**31**:e1707612.
111. Chen F, Hong H, Shi S, Goel S, Valdovinos HF, Hernandez R, et al. Engineering of hollow mesoporous silica nanoparticles for remarkably enhanced tumor active targeting efficacy. *Sci Rep* 2014;**4**:5080.
112. Zhang Z, Ji Y, Chen W. Hollow MnO_2/GNPs serving as a multi-responsive nanocarrier for controlled drug release. *Chin J Chem Eng* 2020;**28**:1405−14.
113. Sun X, He G, Xiong C, Wang C, Lian X, Hu L, et al. One-pot fabrication of hollow porphyrinic MOF nanoparticles with ultrahigh drug loading toward controlled delivery and synergistic cancer therapy. *ACS Appl Mater Interfaces* 2021;**13**:3679−93.
114. Wang X, Li X, Ito A, Yoshiyuki K, Sogo Y, Watanabe Y, et al. Hollow structure improved anti-cancer immunity of mesoporous silica nanospheres *in vivo*. *Small* 2016;**12**:3510−5.
115. Ma MY, Zhu YJ, Li L, Cao SW. Nanostructured porous hollow ellipsoidal capsules of hydroxyapatite and calcium silicate: preparation and application in drug delivery. *J Mater Chem* 2008;**18**: 2722−7.
116. Chen JF, Wang JX, Liu RJ, Shao L, Wen LX. Synthesis of porous silica structures with hollow interiors by templating nanosized calcium carbonate. *Inorg Chem Commun* 2004;**7**:447−9.
117. Fuchigami K, Taguchi Y, Tanaka M. Preparation of hemispherical hollow silica microcapsules with different affinity surface by using spherical vaterite calcium carbonate as template. *Polym Adv Technol* 2007;**18**:946−52.
118. Chen JF, Ding HM, Wang JX, Shao L. Preparation and characterization of porous hollow silica nanoparticles for drug delivery application. *Biomaterials* 2004;**25**:723−7.
119. Yang YJ, Tao X, Hou Q, Chen JF. Fluorescent mesoporous silica nanotubes incorporating CdS quantum dots for controlled release of ibuprofen. *Acta Biomater* 2009;**5**:3488−96.
120. Zhao Y, Lin LN, Lu Y, Chen SF, Dong L, Yu SH. Templating synthesis of preloaded doxorubicin in hollow mesoporous silica nanospheres for biomedical applications. *Adv Mater (Weinheim, Ger)* 2010;**22**:5255−9.
121. Guo YJ, Wang YY, Chen T, Wei YT, Chu LF, Guo YP. Hollow carbonated hydroxyapatite microspheres with mesoporous structure: hydrothermal fabrication and drug delivery property. *Mater Sci Eng C* 2013;**33**:3166−72.
122. Dong Z, Feng L, Hao Y, Li Q, Chen M, Yang Z, et al. Synthesis of $CaCO_3$-based nanomedicine for enhanced sonodynamic therapy *via* amplification of tumor oxidative stress. *Chem* 2020;**6**:1391−407.
123. Kittitheeranun P, Sajomsang W, Phanpee S, Treetong A, Wutikhun T, Suktham K, et al. Layer-by-layer engineered nanocapsules of curcumin with improved cell activity. *Int J Pharm* 2015;**492**: 92−102.
124. Park M, Go H, Kim J-H, Rhee SW. Synthesis of hollow silver spheres using spherical vaterite-type calcium carbonate as template. *Bull Kor Chem Soc* 2016;**37**:269−75.

125. Dong Z, Feng L, Hao Y, Chen M, Gao M, Chao Y, et al. Synthesis of hollow biomineralized $CaCO_3$-polydopamine nanoparticles for multimodal imaging-guided cancer photodynamic therapy with reduced skin photosensitivity. *J Am Chem Soc* 2018;**140**:2165−78.
126. Dey N, Santhiya D, Das A. Bio-inspired synthesis of hollow mesoporous bioactive glass nanoparticles using calcium carbonate as solid template. *ChemistrySelect* 2022;**7**:e202200392.
127. Nakashima Y, Takai C, Razavi-Khosroshahi H, Suthabanditpong W, Fuji M. Synthesis of ultra-small hollow silica nanoparticles using the prepared amorphous calcium carbonate in one-pot process. *Adv Powder Technol* 2018;**29**:904−8.
128. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: current treatment and a collaborative pathway to success. *J Clin Oncol* 2015;**33**:3029−35.
129. Orditura M, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM, et al. Treatment of gastric cancer. *World J Gastroenterol* 2014;**20**:1635−49.
130. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat Biotechnol* 2015;**33**:941−51.
131. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res* 1986;**46**:6387−92.
132. Perrault SD, Walkey C, Jennings T, Fischer HC, Chan WCW. Mediating tumor targeting efficiency of nanoparticles through design. *Nano Lett* 2009;**9**:1909−15.
133. Oude Blenke E, Mastrobattista E, Schiffelers RM. Strategies for triggered drug release from tumor targeted liposomes. *Expet Opin Drug Deliv* 2013;**10**:1399−410.
134. Cabral H, Matsumoto Y, Mizuno K, Chen Q, Murakami M, Kimura M, et al. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. *Nat Nanotechnol* 2011;**6**:815−23.
135. Chauhan VP, Stylianopoulos T, Martin JD, Popović Z, Chen O, Kamoun WS, et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. *Nat Nanotechnol* 2012;**7**:383−8.
136. Soo Choi H, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, et al. Renal clearance of quantum dots. *Nat Biotechnol* 2007;**25**:1165−70.
137. Ju C, Mo R, Xue J, Zhang L, Zhao Z, Xue L, et al. Sequential intracellular nanoparticle delivery system for deep tumor penetration. *Angew Chem Int Ed* 2014;**53**:6253−8.
138. Wong C, Stylianopoulos T, Cui J, Martin J, Chauhan VP, Jiang W, et al. Multistage nanoparticle delivery system for deep penetration into tumor tissue. *PANS (Pest Artic News Summ)* 2011;**108**:2426−31.
139. Wang X, Zhang M, Zhang L, Li L, Li S, Wang C, et al. Designed synthesis of lipid-coated polyacrylic acid/calcium phosphate nanoparticles as dual pH-responsive drug-delivery vehicles for cancer chemotherapy. *Chem Eur J* 2017;**23**:6586−95.
140. Ishida T, Harashima H, Kiwada H. Liposome clearance. *Biosci Rep* 2002;**22**:197−224.
141. Gillies RJ, Robey I, Gatenby RA. Causes and consequences of increased glucose metabolism of cancers. *J Nucl Med* 2008;**49**(Suppl 2):24S−42S.
142. Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. *Nat Rev Drug Discov* 2011;**10**:767−77.
143. Xu D, Jin T, Song L, Zhu N, Han L, Hong H. Synthesis of stable calcium carbonate nanoparticles for pH-responsive controlled drug release. *Mater Lett* 2023;**333**:133635.
144. Bohn T, Rapp S, Luther N, Klein M, Bruehl TJ, Kojima N, et al. Tumor immunoevasion *via* acidosis-dependent induction of regulatory tumor-associated macrophages. *Nat Immunol* 2018;**19**:1319−29.
145. Maleki Dizaj S, Barzegar-Jalali M, Zarrintan MH, Adibkia K, Lotfipour F. Calcium carbonate nanoparticles as cancer drug delivery system. *Expet Opin Drug Deliv* 2015;**12**:1649−60.
146. Wang M, Zhou B, Wang L, Zhou F, Smith N, Saunders D, et al. Biodegradable pH-responsive amorphous calcium carbonate nanoparticles as immunoadjuvants for multimodal imaging and enhanced photoimmunotherapy. *J Mater Chem B* 2020;**8**:8261−70.
147. Shi H, Li L, Zhang L, Wang T, Wang C, Zhu D, et al. Designed preparation of polyacrylic acid/calcium carbonate nanoparticles with high doxorubicin payload for liver cancer chemotherapy. *CrystEngComm* 2015;**17**:4768−73.
148. Cheng C, Zhang X, Meng Y, Chen L, Zhang Q. Development of a dual drug-loaded hydrogel delivery system for enhanced cancer therapy: *in situ* formation, degradation and synergistic antitumor efficiency. *J Mater Chem B* 2017;**5**:8487−97.
149. Wei J, Cheang T, Tang B, Xia H, Xing Z, Chen Z, et al. The inhibition of human bladder cancer growth by calcium carbonate/CaIP6 nanocomposite particles delivering AIB1 siRNA. *Biomaterials* 2013;**34**:1246−54.
150. Nie B, Wang H, Rao C, Zhang Y, Wang H, Lian X, et al. Preparation and characterization of sodium alginate/phosphate-stabilized amorphous calcium carbonate nanocarriers and their application in the release of curcumin. *Nanotechnology* 2021:32.
151. Chen S, Zhao D, Li F, Zhuo RX, Cheng SX. Co-delivery of genes and drugs with nanostructured calcium carbonate for cancer therapy. *RSC Adv* 2012;**2**:1820−6.
152. Chiang PH, Fan CH, Jin Q, Yeh C-K. Enhancing doxorubicin delivery in solid tumor by superhydrophobic amorphous calcium carbonate−doxorubicin silica nanoparticles with focused ultrasound. *Mol Pharm* 2022;**19**:3894−905.
153. Rao C, Li M, Sun X, Li M, Lian X, Wang H, et al. Preparation and characterization of phosphate-stabilized amorphous calcium carbonate nanoparticles and their application in curcumin delivery. *Mater Chem Phys* 2020;**255**:123552.
154. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol* 2013;**31**:51−72.
155. Zheng P, Ding B, Jiang Z, Xu W, Li G, Ding J, et al. Ultrasound-augmented mitochondrial calcium ion overload by calcium nanomodulator to induce immunogenic cell death. *Nano Lett* 2021;**21**:2088−93.
156. Zheng P, Ding B, Zhu G, Li C, Lin J. Biodegradable Ca^{2+} nanomodulators activate pyroptosis through mitochondrial Ca^{2+} overload for cancer immunotherapy. *Angew Chem Int Ed Engl* 2022;**61**:e202204904.
157. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* 2007;**13**:1050−9.
158. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med* 2005;**202**:1691−701.
159. Dudek AM, Garg AD, Krysko DV, De Ruysscher D, Agostinis P. Inducers of immunogenic cancer cell death. *Cytokine Growth Factor Rev* 2013;**24**:319−33.
160. Disis ML, Bernhard H, Jaffee EM. Use of tumour-responsive T cells as cancer treatment. *Lancet* 2009;**373**:673−83.
161. Dai Z, Tang J, Gu Z, Wang Y, Yang Y, Yang Y, et al. Eliciting immunogenic cell death *via* a unitized nanoinducer. *Nano Lett* 2020;**20**:6246−54.
162. An J, Zhang K, Wang B, Wu S, Wang Y, Zhang H, et al. Nanoenabled disruption of multiple barriers in antigen cross-presentation of dendritic cells *via* calcium interference for enhanced chemo-immunotherapy. *ACS Nano* 2020;**14**:7639−50.
163. Xu X, Li R, Dong R, Yang Y, Wang H, Cheng J, et al. *In vitro* characterization and cellular uptake profiles of TAMs-targeted lipid calcium carbonate nanoparticles for cancer immunotherapy. *Acta Mater Med* 2022:1.
164. Kang H, Zhang K, Wong DSH, Han F, Li B, Bian L. Near-infrared light-controlled regulation of intracellular calcium to modulate macrophage polarization. *Biomaterials* 2018;**178**:681−96.

165. Li M, Qin M, Song G, Deng H, Wang D, Wang X, et al. A biomimetic antitumor nanovaccine based on biocompatible calcium pyrophosphate and tumor cell membrane antigens. *Asian J Pharm Sci* 2021;**16**:97−109.
166. Wang S, Ni D, Yue H, Luo N, Xi X, Wang Y, et al. Exploration of antigen induced $CaCO_3$ nanoparticles for therapeutic vaccine. *Small* 2018;**14**:e1704272.
167. Chen Q, Wang C, Zhang X, Chen G, Hu Q, Li H, et al. *In situ* sprayed bioresponsive immunotherapeutic gel for post-surgical cancer treatment. *Nat Nanotechnol* 2019;**14**:89−97.
168. Ni J, Song J, Wang B, Hua H, Zhu H, Guo X, et al. Dendritic cell vaccine for the effective immunotherapy of breast cancer. *Biomed Pharmacother* 2020;**126**:110046.
169. Huang H, Zhang W, Liu Z, Guo H, Zhang P. Smart responsive-calcium carbonate nanoparticles for dual-model cancer imaging and treatment. *Ultrasonics* 2020;**108**:106198.
170. Deshpande N, Needles A, Willmann JK. Molecular ultrasound imaging: current status and future directions. *Clin Radiol* 2010;**65**:567−81.
171. Wilson SR, Burns PN. Microbubble-enhanced US in body imaging: What role? *Radiology* 2010;**257**:24−39.
172. Min KH, Min HS, Lee HJ, Park DJ, Yhee JY, Kim K, et al. pH-Controlled gas-generating mineralized nanoparticles: a theranostic agent for ultrasound imaging and therapy of cancers. *ACS Nano* 2015;**9**:134−45.
173. Dong L, Xu YJ, Sui C, Zhao Y, Mao LB, Gebauer D, et al. Highly hydrated paramagnetic amorphous calcium carbonate nanoclusters as an MRI contrast agent. *Nat Commun* 2022;**13**:5088.
174. Gang X, Wang L, Jia J, Wang H, Lian X, Gao X, et al. Synthesis and biological evaluation of fluorescent hyaluronic acid modified amorphous calcium phosphate drug carriers for tumor-targeting. *Int J Biol Macromol* 2021;**182**:1445−54.
175. Ensrud KE. Epidemiology of fracture risk with advancing age. *J Gerontol A Biol Sci Med Sci* 2013;**68**:1236−42.
176. Rashki Kemmak A, Rezapour A, Jahangiri R, Nikjoo S, Farabi H, Soleimanpour S. Economic burden of osteoporosis in the world: a systematic review. *Med J Islam Repub Iran* 2020;**34**:154.
177. Cui L, Jackson M, Wessler Z, Gitlin M, Xia W. Estimating the future clinical and economic benefits of improving osteoporosis diagnosis and treatment among women in China: a simulation projection model from 2020 to 2040. *Arch Osteoporosis* 2021;**16**:118.
178. Battafarano G, Rossi M, De Martino V, Marampon F, Borro L, Secinaro A, et al. Strategies for bone regeneration: from graft to tissue engineering. *Int J Mol Sci* 2021;**22**:1128.
179. Wang X, Ackermann M, Wang S, Tolba E, Neufurth M, Feng Q, et al. Amorphous polyphosphate/amorphous calcium carbonate implant material with enhanced bone healing efficacy in a critical-size defect in rats. *Biomed Mater* 2016;**11**:035005.
180. Deng Y, Wei W, Tang P. Applications of calcium-based nanomaterials in osteoporosis treatment. *ACS Biomater Sci Eng* 2022;**8**:424−43.
181. Zhang J, Chen H, Zhao M, Liu G, Wu J. 2D nanomaterials for tissue engineering application. *Nano Res* 2020;**13**:2019−34.
182. Stevens MM, Mecklenburg G. Bio-inspired materials for biosensing and tissue engineering. *Polym Int* 2012;**61**:680−5.
183. Tolba E, Müller WEG, Abd El-Hady BM, Neufurth M, Wurm F, Wang S, et al. High biocompatibility and improved osteogenic potential of amorphous calcium carbonate/vaterite. *J Mater Chem B* 2016;**4**:376−86.
184. Opitz P, Besch L, Panthöfer M, Kabelitz A, Unger RE, Emmerling F, et al. Insights into the *in vitro* formation of apatite from Mg-stabilized amorphous calcium carbonate. *Adv Funct Mater* 2021; **31**:2007830.
185. Merle M, Soulié J, Sassoye C, Roblin P, Rey C, Bonhomme C, et al. Pyrophosphate-stabilised amorphous calcium carbonate for bone substitution: toward a doping-dependent cluster-based model. *CrystEngComm* 2022;**24**:8011−26.
186. Tolba E, Wang X, Wang S, Neufurth M, Ackermann M, Schröder HC, et al. Amorphous polyphosphate and ca-carbonate nanoparticles improve the self-healing properties of both technical and medical cements. *Biotechnol J* 2020;**15**:2000101.
187. Luo H, Benner R, Long RA, Hu J. Subcellular localization of marine bacterial alkaline phosphatases. *Proc Natl Acad Sci U S A* 2009;**106**: 21219−23.
188. Lorenz B, Schröder HC. Mammalian intestinal alkaline phosphatase acts as highly active exopolyphosphatase. *Biochim Biophys Acta* 2001;**1547**:254−61.
189. Zhang K, Zhou Y, Xiao C, Zhao W, Wu H, Tang J, et al. Application of hydroxyapatite nanoparticles in tumor-associated bone segmental defect. *Sci Adv* 2019;**5**:eaax6946.
190. Straub DA. Calcium supplementation in clinical practice: a review of forms, doses, and indications. *Nutr Clin Pract* 2007;**22**:286−96.
191. Vaisman N, Shaltiel G, Daniely M, Meiron OE, Shechter A, Abrams SA, et al. Increased calcium absorption from synthetic stable amorphous calcium carbonate: double-blind randomized crossover clinical trial in postmenopausal women. *J Bone Miner Res* 2014;**29**:2203−9.
192. Cai M, Yang L, Zhang S, Liu J, Sun Y, Wang X. A bone-resorption surface-targeting nanoparticle to deliver anti-miR214 for osteoporosis therapy. *Int J Nanomed* 2017;**12**:7469−82.
193. Gao C, Wei D, Yang H, Chen T, Yang L. Nanotechnology for treating osteoporotic vertebral fractures. *Int J Nanomed* 2015;**10**: 5139−57.
194. Yu W, Sun TW, Qi C, Ding Z, Zhao H, Chen F, et al. Strontium-doped amorphous calcium phosphate porous microspheres synthesized through a microwave-hydrothermal method using fructose 1,6-bisphosphate as an organic phosphorus source: application in drug delivery and enhanced bone regeneration. *ACS Appl Mater Interfaces* 2017;**9**:3306−17.
195. Wang J, Tao S, Jin X, Song Y, Zhou W, Lou H, et al. Calcium supplement by tetracycline guided amorphous calcium carbonate potentiates osteoblast promotion for synergetic osteoporosis therapy. *Theranostics* 2020;**10**:8591−605.
196. Zhao D, Liu CJ, Zhuo RX, Cheng SX. Alginate/$CaCO_3$ hybrid nanoparticles for efficient codelivery of antitumor gene and drug. *Mol Pharm* 2012;**9**:2887−93.
197. Hoffman JR, Ben-Zeev T, Zamir A, Levi C, Ostfeld I. Examination of amorphous calcium carbonate on the inflammatory and muscle damage response in experienced resistance trained individuals. *Nutrients* 2022;**14**:1894.
198. Wang S, Neufurth M, Schepler H, Tan R, She Z, Al-Nawas B, et al. Acceleration of wound healing through amorphous calcium carbonate, stabilized with high-energy polyphosphate. *Pharmaceutics* 2023;**15**: 494.
199. Tewes F, Gobbo OL, Ehrhardt C, Healy AM. Amorphous calcium carbonate based-microparticles for peptide pulmonary delivery. *ACS Appl Mater Interfaces* 2016;**8**:1164−75.
200. Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, et al. Analysis of nanoparticle delivery to tumours. *Nat Rev Mater* 2016;**1**: 16014.
201. Sung JH, Park SJ, Jeong MS, Song KS, Ahn KS, Ryu HR, et al. Physicochemical analysis and repeated-dose 90-days oral toxicity study of nanocalcium carbonate in Sprague-Dawley rats. *Nanotoxicology* 2015;**9**:603−12.
202. Dong Y, Lan T, Wang X, Zhang Y, Jiang L, Sui X. Preparation and characterization of soy protein microspheres using amorphous calcium carbonate cores. *Food Hydrocolloids* 2020;**107**:105953.
203. Qian K, Shi T, Tang T, Zhang S, Liu X, Cao Y. Preparation and characterization of nano-sized calcium carbonate as controlled release pesticide carrier for validamycin against Rhizoctonia solani. *Microchim Acta* 2011;**173**:51−7.
204. Koga N, Nakagoe Y, Tanaka H. Crystallization of amorphous calcium carbonate. *Thermochim Acta* 1998;**318**:239−44.