Review

Functionalized Calcium Carbonate–Based Microparticles as a Versatile Tool for Targeted Cancer Treatment

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Functionalized Calcium Carbonate–Based Microparticles as a Versatile Tool for Targeted Cancer Treatment

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Abstract: Nano- and microparticles are increasingly widely used in biomedical research and applications, particularly as specific labels and targeted delivery vehicles. Silica has long been considered the best material for such vehicles, but it has some disadvantages limiting its potential, such as the proneness of silica-based carriers to spontaneous drug release. Calcium carbonate (CaCO3) is an emerging alternative, being easily available, cost-effective, biocompatible material with high porosity and surface reactivity, which makes it an attractive choice for targeted drug delivery. CaCO3 particles are used in this field in the form of either bare CaCO3 microbeads or core/shell microparticles representing polymer-coated CaCO3 cores. In addition, they serve as removable templates for obtaining hollow polymer microcapsules. Each of these types of particles has its specific advantages in terms of biomedical applications. CaCO3 microbeads are primarily used due to their capacity for carrying pharmaceutics, whereas core/shell systems ensure better protection of the drug-loaded core from the environment. Hollow polymer capsules are particularly attractive because they can encapsulate large amounts of pharmaceutical agents and can be so designed as to release their contents in the target site in response to specific stimuli. This review focuses first on the chemistry of the CaCO3 cores, core/shell microbeads, and polymer microcapsules. Then, systems using these structures for the delivery of therapeutic agents, including drugs, proteins, and DNA, are outlined. The results of systematic analysis of available data are presented. They show that the encapsulation of various therapeutic agents in CaCO3-based microbeads or polymer microcapsules is a promising technique of drug delivery, especially in cancer therapy, enhancing drug bioavailability and specific targeting of cancer cells while reducing side effects. To date, research in CaCO3-based microparticles and polymer microcapsules assembled on CaCO3 templates has mainly dealt with their properties *in vitro*, whereas their *in vivo* behavior still remains poorly studied. However, an enormous potential of these highly biocompatible carriers for in vivo applications is undoubted. This last issue is addressed in depth in the Conclusion and Outlook sections of the review.

Keywords: calcium carbonate; microparticles; microcapsules; core/shell structures; targeted delivery; anticancer treatment

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1. Introduction

Microparticles are widely used in various fields of research and drug delivery applications [1,2]. Among the various materials used for microparticle fabrication, silica has long been considered the best candidate, but it has several disadvantages that limit its clinical potential, especially in preventing the spontaneous drug release [3]. Calcium carbonate ($CaCO_3$) is an abundant, inexpensive, biocompatible material with suitable chemical and physical properties, such as a small size of the particles with a large surface area [4]. These properties make it an attractive material for numerous biomedical applications and an ideal choice for targeted cancer immunotherapy [5]. There are three polymorphs of $CaCO_3$ particles: calcite, aragonite, and vaterite crystals. Though less thermodynamically stable than the others, vaterite crystals are spherical, composed of nanodomains, and highly porous, which makes them a good candidate for using in drug delivery systems [6,7].

The most common methods of synthesis of $CaCO_3$ microparticles are solid–liquid–gas carbonation [8] and chemical precipitation through the reaction of $CaCl_2$ with Na_2CO_3 in an aqueous medium [9]. There are also other methods of synthesis of $CaCO_3$ microparticles [10], such as the supercritical fluid technology [11] and the emulsion techniques [12,13]. In the course of synthesis, the temperature, pH, reagent concentrations, and other parameters can be controlled to optimize the size, morphology, and composition of the microparticles. It has been shown that gradual addition of a calcium nitrate solution to the sodium carbonate solution allows controlling the saturation of the reaction medium and obtaining smaller $CaCO_3$ particles after prolonged agitation. Overall, temperature influences particle morphology and polymorphism, whereas the calcium and carbonate ion concentrations determine their size [14]. These different techniques of synthesis offer flexible approaches for obtaining $CaCO_3$ particles suitable for various therapeutic applications. $CaCO_3$-based microparticles have a wide range of potential applications, particularly in targeted drug delivery. Their use can offer significant advantages in terms of efficiency, cost-effectiveness, and sustainability compared to existing materials.

Three main types of $CaCO_3$-based microparticles with sizes ranging from about 0.2 to 6 μm have been extensively studied: core-only microparticles, polymer-coated cores (or core/shell microparticles), and hollow (shell) polymer capsules, for which $CaCO_3$ particles are used as sacrificial templates [2,15,16] (Figure 1). Each of these types possesses unique characteristics suitable for specific applications in cancer treatment.

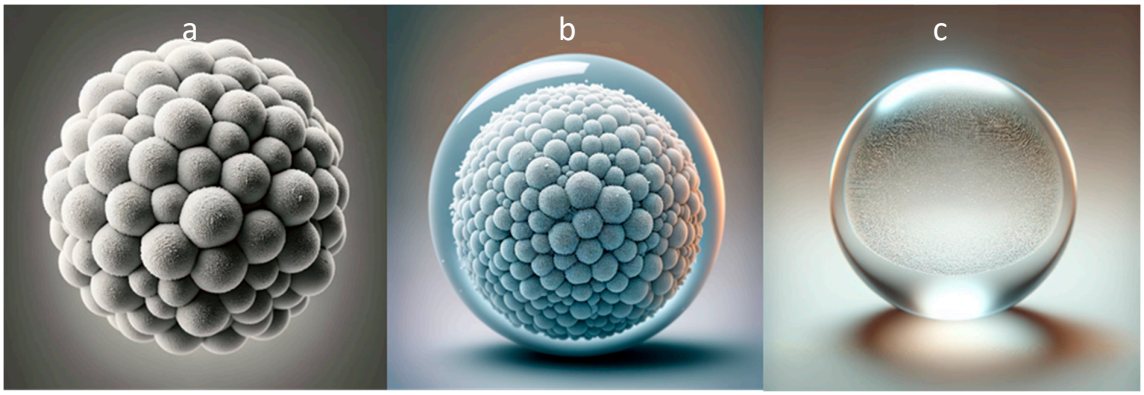

Figure 1. Spherical $CaCO_3$-based microparticles for targeted cancer therapy: (a) a $CaCO_3$ core-only microparticle; (b) a $CaCO_3$ core/shell microparticle; (c) a polyelectrolyte shell–only microcapsule without a core.

Core-only microparticles are primarily used due to their capacity for absorbing and carrying therapeutic agents. Their simple, porous structure also ensures drug release. However, their use is limited by the lack of targeting specificity and insufficient resistance to potentially aggressive factors of biological microenvironment. Additional strategies may be necessary to prevent their degradation or aggregation during the delivery [17].

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Core/shell structures are considerably more advantageous, because their polyelectrolyte shell provides enhanced protection of the encapsulated compound compared to core-only systems and can be functionalized to ensure specific targeting. Current research focuses on developing new strategies to enhance stability, targeting, and release control by coating microparticles with polymers [18] or lipids [19]. These microparticles can be designed to respond to specific stimuli, such as changes in pH [20,21], or temperature [22], by releasing their contents. They are commonly fabricated by means of layer-by-layer (LbL) deposition of alternating anionic and cationic polyelectrolytes, depending on the charge of the template microparticle [23,24].

Polymer microcapsules [16,25] are particularly interesting because of their capacity for encapsulating therapeutic agents while avoiding the adverse effect of $CaCO_3$ on the cellular calcium balance. They also can be designed to respond to specific stimuli, allowing for targeted drug release within tumors [26,27]. Polymeric microcapsules are synthesized on the basis of $CaCO_3$ templates, which are usually dissolved by ethylenediaminetetraacetic acid (EDTA) after LbL assembly of polyelectrolytes [28]. The EDTA concentration determines the dissolution rate and the final properties of the microparticles, including size, porosity, and stability.

Various therapeutic agents, including low-molecular-weight drugs, proteins, and nucleic acids, can be encapsulated by loading into $CaCO_3$ cores through absorption or chemical coprecipitation during the formation of the cores [29]. The loading capacity of these systems depends on several factors, such as the porosity and specific surface area of the $CaCO_3$ particles and the chemical properties of the drug. Studies have shown significant effectiveness of low-molecular-weight drug encapsulation [30] and their controlled release from $CaCO_3$ cores [31], sometimes with a reduced cytotoxicity [32]. The efficiency of encapsulation and stability of encapsulated molecules have been also demonstrated for proteins [33] and nucleic acids [34]. Drug release from delivery systems based on $CaCO_3$ microparticles can be activated by external stimuli, such as a change in pH [35] (slightly acidic in tumors) or temperature [22]. For targeted drug delivery, $CaCO_3$ microparticles can be functionalized with recognition molecules, usually antibodies, interacting with specific receptors on target cells [36]. Moreover, *in vivo* studies of a nasal drug delivery system based on $CaCO_3$ microparticles has shown improved bioavailability of the active substance [37]. Recently, *in vivo* applications of $CaCO_3$ particles using various administration routes have been intensely studied and proven to be promising [38].

In conclusion, the loading of drugs into calcium carbonate cores, core/shell microparticles based on them, or microcapsules is a promising technique in the field of drug delivery, especially for cancer therapy. $CaCO_3$-based microparticles efficiently encapsulate various therapeutic agents, improving their bioavailability and specifically targeting cancer cells while reducing side effects. In this review, we will first discuss the methods of synthesis of calcium carbonate cores and fabrication of $CaCO_3$-based microparticles and microcapsules, then explore the systems for delivery small-molecule drugs, proteins, and DNAs based on each of these structures, and finally address the potential uses and key challenges of these microstructures in cancer treatment.

2. Core-Only $CaCO_3$ Microparticles

Calcium carbonate cores have been used as containers over the past two decades [39] and offer numerous advantages for the delivery of pharmacological compounds, such as biocompatibility, a high loading capacity, and maintenance of the properties of the loaded molecules [40]. Their size and shape vary depending on synthesis conditions, including temperature, reactant concentrations, viscosity of the medium, and reaction time, which allows obtaining cores with desired properties [1,6,7]. The internal porous structure of functionalized calcium carbonate cores is also an important factor influencing drug loading, which has recently been elucidated by mercury intrusion porosimetry and scanning electron microscopy with a focused ion beam [41]. A reduced pore size has been found to be associated with an increased maximum payload, i.e., a higher capacity for retaining compounds within the particles.

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2.1. Loading Methods

Calcium carbonate cores are used for the loading of small molecules [21,42], proteins [43,44], nucleic acids [34], and radionuclides [45,46]. The substances are loaded into the $CaCO_3$ cores either by co-synthesis, when the proteins are captured by the $CaCO_3$ cores during their growth, or by adsorption of loading molecules onto the matrix surface of preformed $CaCO_3$ cores [47]. An alternative drug loading method by solvent evaporation is suitable for small molecules with different solubilities [42]. The adsorption of poorly soluble drugs onto the $CaCO_3$ particles may help overcome the low bioavailability of drugs [48], whereas loading during co-synthesis leads to aggregation of proteins [43]. The co-precipitation method has proven to have a high loading efficiency for both small-molecule drugs and proteins [18,49]. The loading efficiency depends on the drug diffusion through the pores at the pH and ionic strength suited to each particular compound, while ensuring preservation of its bioactivity. For example, the loading of superoxide dismutase into vaterite $CaCO_3$ crystals at pH 8.5 was highly efficient, with its activity retained, whereas at pH 9.5, only a 30% retention was achieved [43].

Enhancement of protein encapsulation into 6.9 μm $CaCO_3$ microparticles using protein–polysaccharide interactions has been shown [50]. The chitin-binding domain (ChBD) was inserted into a β-lactamase protein (BlaP) to obtain a chimeric protein, BlaPChBD, exhibiting affinity for hyaluronic acid (HA). In the presence of HA, the particle size was decreased to 4.5 μm, which indicated a templating effect of HA on $CaCO_3$. The chitin-binding domain (ChBD) ensured a more stable interaction between the protein and HA, reducing aggregation and decreasing the particle size. The use of supercritical CO_2 ($ScCO_2$) technology in the presence of HA ensured successful encapsulation of BlaPChBD in vaterite $CaCO_3$ microparticles, increasing protein encapsulation sixfold compared to BlaP alone. In addition, thrombin cleavage sites were introduced to facilitate protein release by protease cleavage, the release rate being increased from less than 20% to 87% within 36 h. The β-lactamase encapsulation rate was below 1%, apparently, due to unfavorable electrostatic interactions at pH 6.5, and was slightly increased (to 1.2%) after the insertion of the chitin-binding domain. The use of HA significantly increased the encapsulation of BlaPChBD (to 6.27%) due to protein–polysaccharide interactions. The results demonstrate the efficacy of using HA for enhancing encapsulation and controlled release of proteins in $CaCO_3$-based delivery systems, offering a promising approach to the development of biodegradable and targeted drug delivery systems.

2.2. Demonstration and Limitations

Loading of three therapeutic proteins (insulin, catalase, and aprotinin) into vaterite $CaCO_3$ cores has shown that the protein loading capacity is independent of their molecular weight and depends only on inter-protein interactions [44]. The tested proteins differ from one another in adsorption kinetics, which indicates differences in the adsorption mechanisms.

The efficiencies of loading catalase into $CaCO_3$ vaterite crystals by means of absorption into preformed crystals (ADS) and co-synthesis (COS) [51] have been compared. COS has been shown to be more efficient, as in the case of the loading of small molecules [18], with a protein content of 20.3% versus 3.5% loaded by the ADS method. The high loading capacity of COS, with a local protein concentration of about 550 mg/mL, was due to $CaCl_2$-induced interprotein interactions resulting in aggregation. The adsorption isotherms better fitted the Langmuir and Brunauer–Emmett–Teller (BET) models than the Freundlich model, which indicated aggregation in solution followed by absorption of aggregates into the crystals. Furthermore, catalase was found to retain about 79% of its specific activity after ADS loading. The stability of the aggregates in the crystals was confirmed by that catalase loaded by the COS method could not be effectively removed by a single washing, unlike catalase loaded by the ADS method. This study highlights the high potential of the COS method for loading large amounts of active proteins into $CaCO_3$ crystals, offering a new approach to the encapsulation of therapeutic proteins [51]. One of the main problems with vaterite $CaCO_3$ particles is their aggregation [25]. However, stabilizers, such as SDS, successfully overcome this problem [21].

The $CaCO_3$-based delivery systems are often designed to be pH-dependent. Calcium carbonate/hyaluronate/glutamate submicron hollow spheres loaded with doxorubicin (DOX) [52]

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released 59.97% of DOX within 14 days at pH 7.4, 87.89% at pH 6.0, and 99.15% at pH 5.0, with a loading efficiency of 85%. Specific binding of these particles to cancer cells was provided by the ligand–receptor interaction between HA and CD44 receptors, overexpressed on cancer cells. The IC_{50} of DOX-loaded microspheres was much lower than that of free DOX when tested on HeLa cancer cells. At the same time, in tests on V79-4 normal cells, the IC_{50} was significantly lower for free DOX than for DOX-loaded microspheres, thus confirming the enhanced specificity of treatment with microspheres (Figure 2).

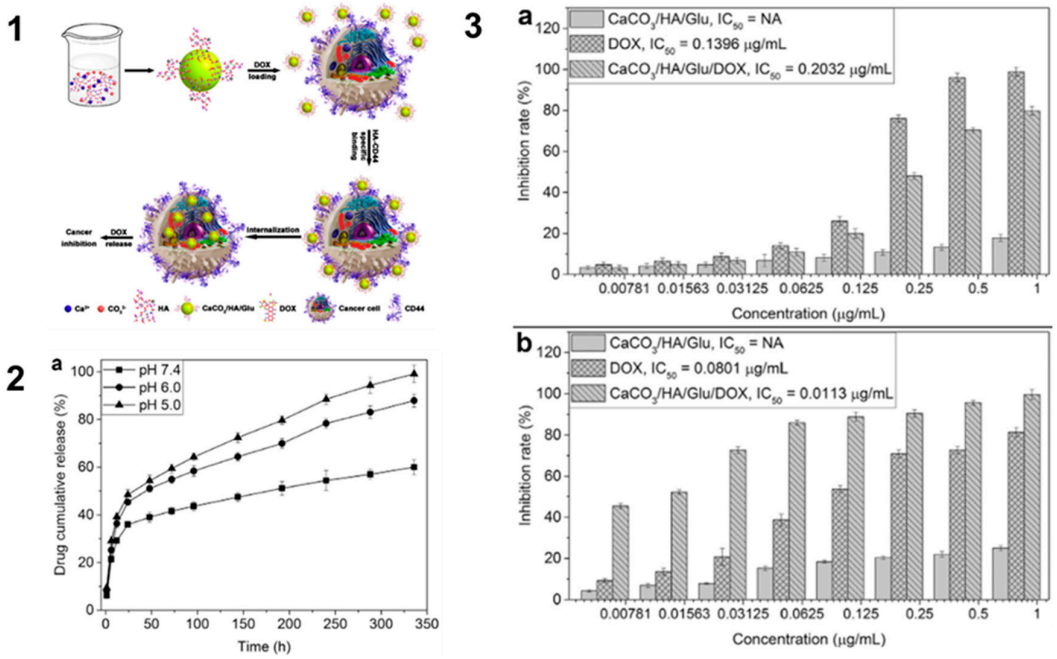

Figure 2. (**1**) Preparation of $CaCO_3$/HA/Glu MHSs, efficient loading of DOX, targeted delivery, specific internalization, and significant inhibition of cancer cells. (**2**) *In vitro* release profiles of $CaCO_3$/HA/Glu/DOX under different pH. Each data represents the mean ± S.D., n = 3. (**3**) Cytotoxic effects of free DOX, CaCO3/HA/Glu, and $CaCO^3$/HA/Glu/DOX on (**a**) V79-4 and (**b**) HeLa cells after 3-d treatment. Each data represents the mean ± S.D., n = 3. Abbreviations: HA, hyaluronate; Glu, glutamate; MHSs, mesoporous hollow spheres; DOX, doxorubicin. Adapted with permission from Guo, Y. et al., J. Coll. Interf. Sci.; published by Elsevier, 2017 [52].

Pneumolysin (PLY)-loaded CaCO3 particles (0.95 μm) containing ovalbumin as a model antigen have been developed as a multimodal antigen delivery system for antitumor vaccines. OVA/CaCO3/PLY nanoparticles obtained by physical adsorption of OVA and PLY on CaCO3 promoted lysosomal degradation, cytoplasmic release, and cross-presentation of antigens, enhancing cellular immunity. The OVA/CaCO3/PLY system induced efficient lysosomal leakage and cytoplasmic delivery of OVA *in vitro* [53].

The kinetics of drug release from the systems based on CaCO3 cores is often bimodal, with initially rapid release due to the dissolution of aggregates followed by sustained release [54]. As the vaterite crystals destabilize, their morphology changes into the calcite one, making the release irreversible. The presence of aggregates within the matrix and the high loading rate by the co-synthesis method, especially for proteins, indicate the limitations of the application of the loading method by adsorption [55]. Nevertheless, other CaCO3-based particle systems are being developed and exhibit a high efficiency in substance delivery. Table 1 summarizes the characteristics of the systems based on CaCO3 cores as vehicles for the delivery of small molecules, proteins, DNAs, and radionuclides.

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3. CaCO3-Based Core/Shell Systems

3.1. Methods of Fabrication

The CaCO3 particles coated with a polyelectrolyte shell are better suited for the delivery of drugs and proteins. Polyelectrolytes are deposited onto the cores by the LbL method [56,57] or by electrospray [58]. Variation of the number of cationic/anionic bilayers deposited on the particle surface allows better control of the kinetics of substance delivery to the target. Application of these polymers is driven by electrostatic interaction, through covalent or hydrogen bonds, which explains how the release of loaded molecules can be induced by different stimuli, such as pH, ionic strength, temperature change, or ultrasound.

For encapsulation of therapeutic agents, adsorption or co-synthesis can be used, which determines their location: on the surface, between the layers, or within the matrix. Figure 3 summarizes the data on fabrication of CaCO3 core-only and core/shell microparticles.

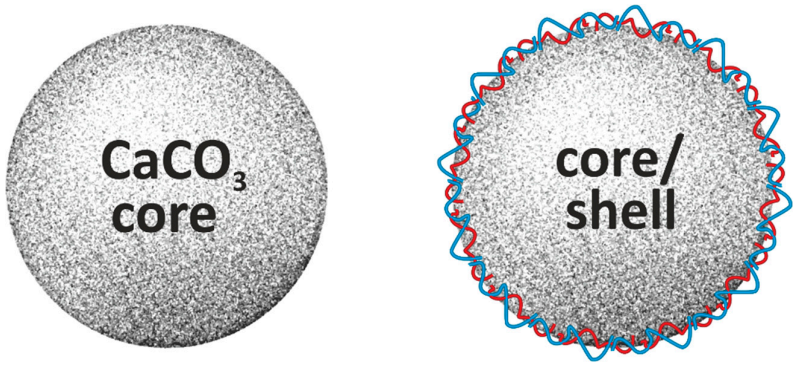

Figure 3. Summary of data on fabrication of porous CaCO3 core-only and CaCO3 core/shell microparticles. **On the left:** The pores size diameters for differently fabricated CaCo3 cores were shown to be in the ranges of 2-50 nm [21], 5-30 nm [43], 10-60 nm [53] or 20-500 nm [49]. **On the right:** The shells on the CaCO3 cores may be fabricated by the deposition of different polymers such as polyl-ornithine/fucoidan [54]; poly(ethylene glycol)/oleic acid [55] ; hyaluronic acid/glutamate [56]; hyaluronic acid/tannic acid [57]; ovalbumin/platelet lysate [58]; poly(diallyldimethylammonium chloride)/poly(sodium 4-styrenesulfonate) [59]; hyaluronic acid [60] ; polylactic acid [61]; poly(acrylic acid) [62].

3.2. Delivery of Small Molecules

In the development of systems for small-molecule delivery based on microparticles, DOX is often used as a model anticancer drug. Efficient loading of DOX has been shown for core/shell microparticles composed of ~2-μm CaCO3 cores coated with poly-L-ornithine and fucoidan. The release of DOX from these particles was confirmed by a significant antiproliferative effect on MCF-7 breast cancer cells [59]. DOX-loaded CaCO3 microparticles modified with oleic acid (OA) and polyethylene glycol (PEG) exhibited a 70% drug release within 2 h in cancer cells in response to specific environment, whereas their stability and drug retention in various other aqueous media were enhanced. Hybrid CaCO3 microspheres have also been obtained using yeast cells as the organic matrix and the polyelectrolytes poly(diallyldimethylammonium chloride) (PDDA) and sodium poly(styrene sulfonate) (PSS) as shell components, with subsequent calcination and DOX loading [60]. Drug release tests showed accelerated release of DOX in an acidic environment (pH 4.8) typical of cancer tissues compared with a neutral medium (pH 7). Cytotoxicity tests have shown a good biocompatibility of CaCO3 microparticles 3 μm in diameter loaded with herbal medicinal products (HMPs) (Figure 4). Gradual decomposition of the coated particles in the acidic microenvironment of tumors ensures the targeted release of the drug directly into the cancer cells, thereby improving the efficacy of the treatment and minimizing the side effects on the surrounding healthy tissue. Thus, the feasibility of delivery of small molecules using the core/shell system has been demonstrated.

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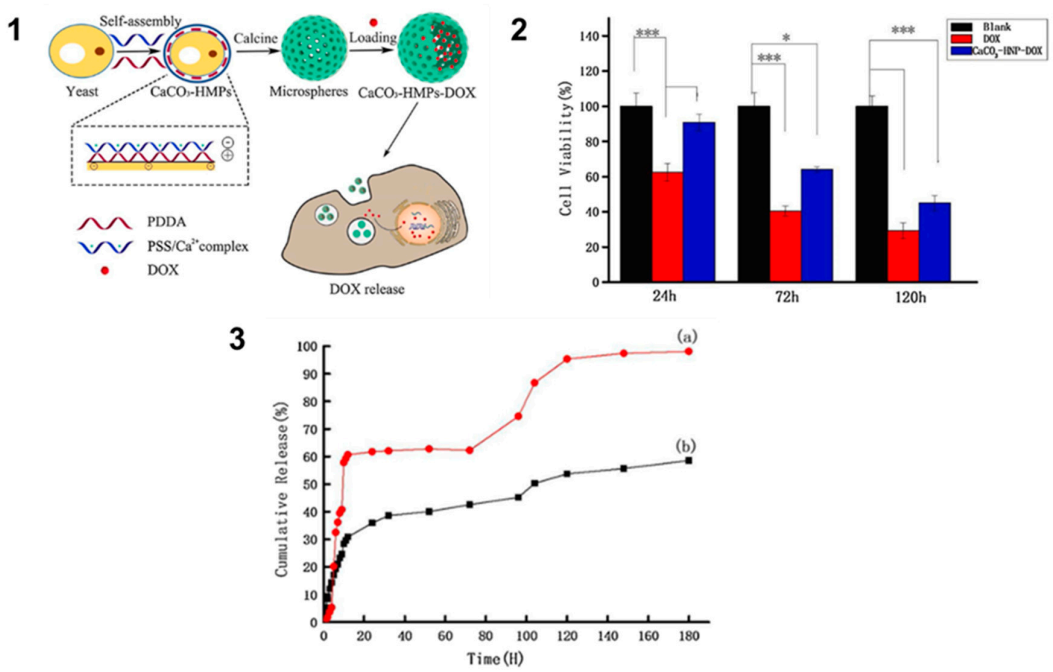

Figure 4. (**1**) Assembly schematic: the preparation of CaCO3-HMPs through self-assembly of two oppositely charged polyelectrolytes, PDDA and PSS, on the surface of yeast cells, as dual templates for drug loading and release. (**2**) Cytotoxicity tests of CaCO3-HMPs, DOX, and the $CaCO_3$-HMPs-DOX drug-delivery system (*P < 0.05, ***P < 0.001); (**3**) Cumulative release curve of adriamycin in different environment: (a) pH = 4.8 and (b) pH = 7. Abbreviations: HMPs, herbal medicinal products; PDDA, poly(diallyldimethylammonium chloride); PSS, poly(sodium 4-styrenesulfonate); DOX, doxorubicin. Reproduced with permission from Wei, Y., et al. Coll. Surf. B Biointerf.; published by Elsevier, 2021. [63].

3.3. Delivery of Proteins

Calcium carbonate microparticles containing cancer cell lysate and coated with polymer substituted with the low-molecular-weight TLR7/8 agonist have been developed, which could serve as novel personalized anticancer vaccines [64].

Solid-in-oil-in-water emulsion method for the manufacture of $CaCO_3$/polylactic acid core/shell microparticles about 1.11 μm in size have been designed as tools for the controlled transport and release of water-soluble bioactive molecules. This technology could be used for developing more effective drug delivery systems [65].

The biomimetic approach has been used to obtain core/shell microparticles with a liquid core consisting of charged emulsion droplets or liposomes and a $CaCO_3$ shell, which also can be used as delivery vehicles [66].

Overall, these techniques improve the encapsulation and release of proteins, offering promising advances for drug delivery systems.

3.4. Delivery of Nucleic Acids

Although encapsulation of nucleic acids in core/shell systems has not yet been reported, some studies envisage it. For example, Bewernitz et al. [66] explore the manufacture of liquid-core/solid-shell microcapsules representing $CaCO_3$-coated emulsions and liposomes. These microcapsules, ranging in size from 2 to 10 μm, have been designed for potential applications in controlled release of substances, including DNA molecules. The method relies on the precipitation of $CaCO_3$ to form a shell around emulsion droplets or liposomes. This approach could be used to engineer a promising

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system for the protection and targeted delivery and release of DNA in biomedical applications due to the possibility of controlling the permeability and degradation of the $CaCO_3$ shell.

Applications of $CaCO_3$ core-based core/shell microparticles are summarized in Table 1.

4. CaCO3-Based Hollow Microcapsules

4.1. Methods of Fabrication

Calcium carbonate-based hollow (or shell) microcapsules represent a fascinating area of research in medical nanotechnology, providing unique opportunities for targeted cancer treatment [29]. These microcapsules with encapsulated therapeutic agents are often designed to interact directly with tumors by functionalization of their surface with antibodies, peptides, proteins, hyaluronic acid, or nucleic acids to ensure controlled, targeted drug delivery [67].

The fabrication of these microcarriers is based on the LbL assembly of polyelectrolytes, a technique first tested on metformin particles [24], which allows the construction of multilayer films with nanometric precision by alternating the immersion of a substrate in solutions of polyelectrolytes of opposite charges. $CaCO_3$ cores, whose synthesis was considered above, are used as templates for the fabrication of microcapsules. Then, the cores are dissolved with a chelating agent, e.g., EDTA and washed, and hollow spherical polyelectrolyte capsules are thus formed. Polyelectrolytes in different combinations, such as poly(allylamine hydrochloride) (PAH) and PSS [18,68], PAH and poly(vinyl sulfate) (PVS) [20], chitosan (Chi) and alginate (Alg) [69], HA and PAH/poly-L-lysine (PLL) [70], and poly-L-arginine (pArg) and dextran sulfate (DS) [71], are particularly effective in forming these multilayers on vaterite $CaCO_3$ cores. Detailed comparison of the stabilities, shrinkabilities, and internal structures of capsules made of different biopolymers have been performed [15]. These polymers, selected for their capability for self-assembling, ensure high stability and functionality of the microcapsules, making it possible to modulate their properties, such as solubility, reactivity and biological compatibility, for the purposes of biomedical engineering and formation of protective coatings and sensors [72]. Figure 5 summarizes the methods for obtaining shell microcapsules.

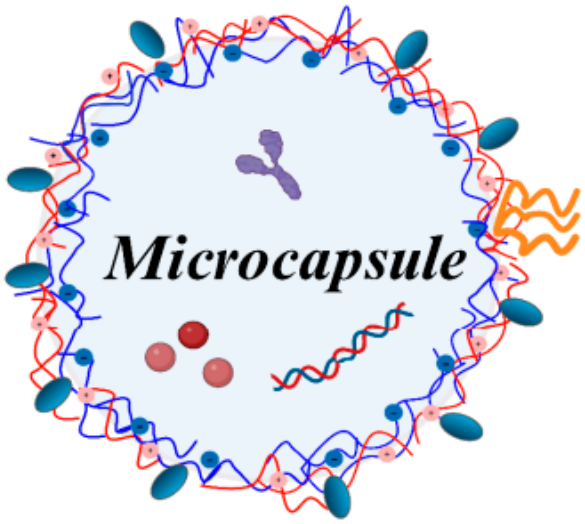

Figure 5. Summary of data on fabrication of microcapsules initially based on the Ca-CO3-microparticles. **PEGylation :** [55,73,74]. **Vectorization :** [75]. Encapsulated Molecules: **Drug :** [76–84] **Protein :** [85–87]

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Nucleic acid : [88–90]. **Shell composition :** poly(allylamine hydrochloride)/poly(sodium 4-styrenesulfonate) [76,79,81,86,87,91,92] ; hyaluronic acid/poly(allylamine hydrochloride), hyaluronic acid/poly-L-lysine [93] ; poly(arginine)/dextran sulfate [77,84,85,90,91] ; polylactic acid/dextran sulfate [83,89] ; poly(allylamine hydrochloride)/dextran sulfate [82,92] ; poly(isopropyl oxazoline)/alginate [78] ; pectin/poly(allylamine hydrochloride) [80] ; poly(methacrylic acid)/poly(N-vinyl-2-pyrrolidone) [88].

4.2. Delivery of Small Molecules

The capability of multilayered polyelectrolyte capsules to host low-molecular-weight drugs for cancer targeting has been recently demonstrated [30]. These smart polymer capsules exhibit considerable versatility, paving the way for future developments in medical nanotechnology and personalized medicine [94]. In recent years, uniformly sized microcapsules obtained on the basis of $CaCO_3$ cores as removable templates, have been loaded with gemcitabine and clodronate [95], DOX [18], apigenin and ascorbic acid [96], curcumin and ciprofloxacin [22], and *Gratiola officinalis* extract [97] as model drugs for cancer and other diseases.

Different encapsulation approaches are used with small-molecule drugs. Microcapsules fabricated using the PAH and PSS polyelectrolytes on $CaCO_3$ cores have exhibited efficiencies of DOX loading by coprecipitation and spontaneous loading of about 73 and 65%, respectively, due to optimized pH and salt concentration [18]. PAH/dextran sulfate (DS) polymer microcapsules designed for the delivery of apigenin and ascorbic acid exhibited a loading efficiency of about 20% for each substance after incubation of the microcapsules in the presence of the drugs [96]. The gemcitabine loading efficiency of submicron pArg/DS microcapsules was about 47% [95].

The microcapsules are so designed as to release the loaded drugs in response to specific stimuli. In the case of PAH/DS capsules containing apigenin and ascorbic acid, *in vitro* release was 45% and 40%, respectively, after 2 h at the physiological pH [96]. This study has also shown that the chemical composition of the capsules strongly affects the drug solubility and rate of its release. The release of DOX by diffusion from PAH/PSS microcapsules was prolonged at pH 6.0 and 7.4, corresponding to the pH values in tumor and normal tissues, respectively. The cumulative release of DOX within 48 h did not exceed 70% [18].

In *in vitro* experiments, pArg/DS microcapsules loaded with gemcitabine were internalized at a rate higher than 75% by macrophages and lung and liver epithelial cells [95]. Experiments in mouse models showed specificity of microcapsule delivery: they were better retained by lung tumor than by healthy lung tissue. The efficiency of encapsulated gemcitabine estimated by the MTT assay was lower than that of the free drug after 24 and 48 h of incubation and equal to it after 72 h of incubation, which confirmed prolonged, gradual release of the drug (Figure 6).

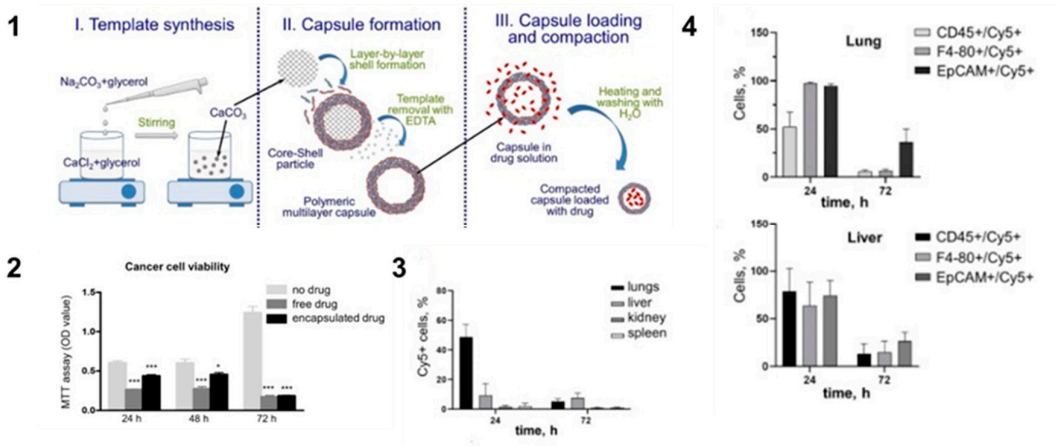

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Figure 6. (**1**) Scheme of the stepwise capsule assembly, compaction, and loading. (**2**) Lung cancer cell viability in the presence of 20 μM free or encapsulated gemcitabine, MTT assay at the indicated time points. (**3**) Number of cells in the lungs, liver, kidney, and spleen with in-ternalized Cy5-labeled capsules relative to the total amount of cells in the respective or-gans 24 and 72 h after intravenous injection of PMC. (**4**) Percentage of hematopoietic cells (CD45+), macrophages (F4/80+), and epithelial cells (EpCAM+) with internalized capsules (Cy5+) in the lungs and liver. Abbreviation: PMC, polymeric multilayer capsules. Adapted with permission from Novoselova, M. V., et al. ACS Appl. Mater. Interfaces; published by American Chemical Society, 2020 [95].

Microcapsules are commonly developed to reduce the side effects of drugs and to allow a more prolonged and targeted action of, e.g., DOX, thereby improving the efficacy of the treatment. It can be co-administered, thus compensating for the rapidity of elimination from the body [32]. Also, the microcapsules containing *Gratiola officinalis* extract were shown to effectively release the drug, causing death of 100% of cultured cancer cells through overcoming protective autophagy [97].

Hollow polymeric microcapsules are also used for encapsulation of live *E. coli* cells. $CaCO_3$ cores containing *E. coli* cells were obtained by co-precipitation and coated with different polyelectrolytes. Then, $CaCO_3$ cores were dissolved in EDTA to obtain capsules with a size of about 5 μm. Encapsulation reduced cell viability, the effect being mainly accounted for by PAH, with only minor contribution of the other components. The encapsulated cells exhibited a prolonged lag phase of growth while retaining the ability to produce green fluorescent protein. About 40% of cells were alive after the encapsulation. This method has potential applications in high-throughput screening of biocatalyst libraries, requiring optimization to improve cell survival [98].

Composite microcapsules based on $CaCO_3$ have been developed that contain various types of pectin with different degrees of methylation and amide content, as well as mixtures of polyelectrolyte complexes, including poly(allylamine) hydrochloride. These $CaCO_3$/pectin capsules were used as matrices for the loading of tetracycline hydrochloride (TCH), with analysis of drug release kinetics using the Higuchi and Korsmeyer–Peppas models. *In vitro* assays demonstrated the influence of $CaCO_3$ polymorphs on the drug release process, with 22–27% of TCH released within 10 h at pH 7.4 [99].

The potential of using $CaCO_3$-templated PAH/PSS polymer capsules for the targeted delivery of vitamin B12 has also been demonstrated [100]. Successful encapsulation of vitamin B12 was confirmed by optical absorption spectroscopy, transmission electron microscopy, and atomic force microscopy data. Experimental data on the specific encapsulation capacity of these polymer capsules for vitamin B12 show their potential as targeted vectors for nutrient delivery, highlighting the effectiveness of the PAH/PSS system in developing biocompatible and stable drug-delivery vectors.

4.3. Delivery of Proteins

Proteins can also be transported and released by polyelectrolyte capsule systems assembled on $CaCO_3$ cores [101]. The chemical methods for the fabrication and post-modification of hollow polymer capsules for proteins delivery, including covalent bonding, electrostatic attachment, and hydrogen bonding, have been described [102]. Proteins can be encapsulated by physical adsorption on preformed $CaCO_3$ cores or by co-precipitation during the $CaCO_3$ particle synthesis. The latter approach has been shown to be five times more efficient [103]. Horseradish peroxidase (HRP) and ovalbumin serving as model antigens have been encapsulated in $CaCO_3$-based pArgt/DS polymer capsules by co-precipitation. After lyophilization in the presence of polyols, HRP retained up to 70% of its enzymatic activity. Ovalbumin-loaded microcapsules were used as model vaccine formulation. Ovalbumin encapsulated in polyelectrolyte microcapsules caused enhanced antigen presentation and amplification of T-cell proliferation compared to soluble ovalbumin. The immunological activity of lyophilized microcapsules was preserved, according to the results of *in vitro* T-cell proliferation assay [104].

The effect of pH on the degradation of polyelectrolyte microcapsules formed on $CaCO_3$ particles with proteins encapsulated by adsorption was also studied [105]. An increase in pH led to an increase in protein yield and PAH detachment, apparently because the acidity of the medium (pH 7) was close

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to the charge exchange point of the PAH amino group. A high concentration of NaCl (2 M) caused considerable PAH dissociation and release of the protein.

4.4. Delivery of Nucleic Acids

Studies using polymeric capsules for delivering genetic material into cells are also carried out. $CaCO_3$-based microcapsules made from biodegradable biopolymers were used for the delivery of all CRISPR-Cas9 components to cells [106]. The efficiency of transfection indicated by loss of red fluorescence in dTomato-expressing HEK293T reached 70%. Submicro- and microcapsules with pArg/DS shells were successfully used as carriers for messenger RNA (mRNA) and small interfering RNA (siRNA) [107]. This study demonstrated that the package efficiency of RNA molecules, delivery efficiency, and biodistribution strongly depended on the size of the capsules. Both studies highlight the importance of developing safe and effective delivery systems for gene therapy and genome editing. The use of microcarriers offers a promising alternative to viral vectors, reducing the associated risks and potentially enhancing the clinical acceptance of these technologies. The delivery systems based on microcapsules are summarized in Table 1.

Finally, the use of $CaCO_3$-based microcapsules in various medical applications, especially in immunotherapy and targeted cancer treatment, appears a promising approach. Ongoing research and innovations in this field could transform cancer treatment, offering more effective and less invasive solutions, notably through the release of small molecules, proteins, and nucleic acids encapsulated in these polyelectrolyte capsules by physical absorption or coprecipitation, thus marking a significant evolution in therapeutic strategies.

5. Conclusion

$CaCO_3$ submicro- and microparticles have a considerable potential as vectors for targeted drug delivery, particularly in cancer treatment. Their controlled dissolution depending on pH ensures targeted drug release in the acidic areas of tumors while maintaining stability in the more neutral circulatory system. Different configurations of the delivery system, core-only and core/shell microparticles and microcapsules, offer solutions for the transport and controlled release of various therapeutic substances, including small molecules, proteins, and nucleic acids.

Vaterite $CaCO_3$ cores are effective for loading small molecules through techniques such as coprecipitation, allowing for subsequent controlled release. However, their rapid degradation *in vivo* can lead to premature release and disrupt cellular calcium balance. To address this issue, core/shell particles have been developed, where the $CaCO_3$ core is coated with a shell of polyelectrolytes, which regulates its degradation, thus allowing sustained and controlled drug release while minimizing cell damage. This system can also be modified to specifically target cells or tissues, improving therapeutic efficacy and reducing side effects.

Finally, $CaCO_3$-based polyelectrolyte capsules overcome the issues entailed with $CaCO_3$ particles. Removal of the core through calcium chelation limits the destabilization of the tumor microenvironment by the increase in intracellular Ca^{2+} and ultimately controlling the pH. The capsules are particularly promising for the encapsulation and controlled release of small molecules, nucleic acids, and proteins, due to their ability to degrade under specific intracellular conditions. Although the delivery of biomacromolecular therapeutic agents presents a huge challenge compared to the delivery of small molecules due to both their high molecular weight and fragile structure, these problems can be solved by using polymer delivery systems [108]. In summary, $CaCO_3$-based particles offer a versatile platform for more effective therapeutic treatments, particularly for complex diseases, such as cancer, due to their adaptability and capability for targeted and controlled drug delivery and release.

6. Outlook: *In Vivo* Studies

6.1. Modulation of the pH of Tumor Environment

Submicron $CaCO_3$ particles offer a promising tool to counteract the characteristic acidity of tumors, a known factor in promoting their aggressiveness and metastatic potential. The targeting of

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tumors with 20- to 300-nm calcium carbonate particles allows gradually increasing the tumor pH to neutrality. This pH modulation is crucial, because a less acidic environment can inhibit the growth and spread of cancer cells, thereby reducing their virulence. Particularly, 100-nm particles stand out for their ability to sustain a prolonged pH elevation. This highlights the importance of the particle size optimization in maximizing the treatment efficacy. Tests on animal models have shown a significant reduction of tumor growth, attesting to the therapeutic potential of this method. However, further research is required to optimize the dosage, evaluate the synergy with other treatments, and predict side effects. This advancement shows a way for improving cancer treatment strategies by targeting a fundamental aspect of tumor biology [109].

6.2. Biodistribution and Biocompatibility

The *in vivo* biodistribution of capsules is a major issue for the development of safe and effective drug carriers. Fluorescent $CaCO_3$-based pArg/DS capsules have been developed for kidney targeting via the renal artery [110]. The high efficiency of delivery to the area of interest was provided by optimization of the administration protocol and dosage.

6.3. Retention, Stability, and Toxicity

$CaCO_3$ particles labeled with ^{224}Ra were proposed for local therapy of disseminated tumors [46]. Biodistribution studies showed that radioactivity was primarily localized in the peritoneal area after administration, with the highest activity associated with intraperitoneal adipose tissue and the parietal peritoneum. The release of ^{224}Ra from the particles was relatively limited, as evidenced by reduced absorption in the skeleton compared to the administration of free ^{224}Ra. Non-abdominal organs, such as the heart, muscles, and brain, displayed radioactivity levels below 100 Bq/g, which indicated a limited radiation exposure outside the abdominal area. These results indicate that radiolabeled $CaCO_3$ particles possess a high retention capacity and targeted bioavailability, making them potentially useful for targeted medical applications, minimizing non-target tissue exposure to radiation. The antitumor effect of $CaCO_3$ microparticles labeled with the alpha-emitting ^{224}Ra was shown in mice [45]. This study highlights the advantage of using $CaCO_3$ as carrier of therapeutic agents and shows a particularly promising therapeutic strategy for tumors located in the abdominal cavity.

$CaCO_3$ core/shell particles 0.8 μm in size were used for encapsulation of the alpha-emitting ^{225}Ac in order to enhance its retention and reduce systemic toxicity during alpha therapy [111]. The study showed a 93–94% retention of ^{225}Ac after 20 days, with the majority of ^{225}Ac-microparticles localized in the lungs, which indicated a reduced renal toxicity potential. *In vivo* tests on Wistar rats confirmed the high retention efficiency of the particles, underscoring the effectiveness of ^{225}Ac-doped core/shell particles in safely retaining alpha emitters used for cancer treatment.

The wide potential applications of $CaCO_3$ nanoparticles in various sectors, including medicine, calls for thorough evaluation of their toxicity. *In vitro* experiments on NIH 3T3 and MCF7 cells treated with $CaCO_3$ nanoparticles at different concentrations (1–50 μg/mL) for 12 to 72 hours showed no cytotoxicity, oxidative stress, or DNA damage, indicating excellent biocompatibility. *In vivo* studies with zebrafish treated with $CaCO_3$ nanoparticles at doses as high as 200 μg/mL showed an absence of significant toxic effects on embryonic development. These results underscore the safety of $CaCO_3$ nanoparticles, suggesting their applicability in medicine and other fields, without cytotoxic or genotoxic risks to biological systems [112].

6.4. Vaccinal Applications

Recent studies illustrate innovative use of vaccines in anticancer immunotherapy, highlighting the *in vivo* efficacy of formulations based on submicron- and micron-sized $CaCO_3$ particles. The physical adsorption of an antigen (ovalbumin) into $CaCO_3$ particles with adsorbed pneumolysin, the key virulence factor of *Streptococcus pneumoniae*, significantly amplified cellular and humoral immunity, demonstrating preventive and therapeutic antitumor efficacy [113]. The 0.95-μm $CaCO_3$

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particles degraded into Ca^{2+} and CO_2 in the acidic lysosomal environment, promoting cross-presentation of antigens. This biodegradability of the particles was confirmed by the detection of intracellular Ca^{2+}, with the highest levels observed for the ovalbumin/$CaCO_3$/pneumolysin group. This study illustrates the induction of a robust immune response, offering an effective platform based on submicron- and micron-sized CaCO3 particles for the development of anticancer immunotherapy through vaccination.

Table 1. $CaCO_3$ core-only and $CaCO_3$-based core/shell microparticles and shell-only microcapsules.

Particle type	Size	Cargo type	Encapsulated molecule	Shell composition	Ref.
Core	1 μm	Small molecule	Doxorubicin	Hyaluronate/glutamate	[52]
Core	0.6–3.2 μm	-	-	-	[6]
Core	0.43 μm	Fluorescent dye	Rhodamine 6G	-	[40]
Core	0.52 μm	-	-	-	[1]
Core	0.4–2.7 μm	-	-	-	[7]
Core	n/a	Protein	BSA	-	[41]
Core	17.9 μm	Small molecule	Ibuprofen, nifedipine, losartan potassium, and metronidazole benzoate	-	[48]
Core	3.1–23.5 μm	Small molecule	Aspirin, vanillin	-	[42]
Core	17.9 μm	Protein	Lysozyme, BSA	-	[49]
Core	3.4 μm	Protein	Superoxide dismutase	-	[43]
Core	10 μm	Protein	Catalase, insulin, aprotinin	-	[44]
Core	0.8-1.6 μm	Small molecule	Doxorubicin	-	[21]
Core	4–5 μm	Protein	Catalase	-	[51]
Core	1 μm	Protein	Ovalbumin, pneumolysin	-	[113]
Core	5.45 μm	Protein	β-lactamase	-	[50]
Core	1.3 μm	-	-	-	[114]
Core	4–7 μm	Radionuclide	^{224}Ra	-	[45]
Core	1–3, 3–15 μm	Radionuclide	^{224}Ra	-	[46]
Core	0.2-1.1 μm	Nucleic acid	DNA	-	[34]
Core/shell	2 μm	Small molecule	Doxorubicin	Poly-L-ornithine/ fucoidan	[115]
Core/shell	0.2 μm	Small molecule	Doxorubicin	Oleic acid/PEG	[116]
Core/shell	3 μm	Small molecule	Doxorubicin	PDDA/PSS	[63]
Core/shell	~10 μm	Protein	Ovalbumin, cancer cell lysate	Poly(HPMA-APMA) with TLR7/8-agonists	[64]
Core/shell	0.65, 3.2 μm	Radionuclide	^{225}Ac	HSA/TA	[111]
Core/shell	~2 μm	Protein	BSA	PLA	[65]
Core/shell	2–4 μm	Fluorescent dye	Nile Red, rhodamine 110	$CaCO_3$	[66]
Core/shell, shell	2–2.5 μm	Small molecule	Doxorubicin	PAH/PSS/QD	[18]
Shell	4.75 μm	Protein	Lactalbumine, lysozyme, horseradish peroxidase, chymotrypsin	-	[39]
Shell	5.4 μm	-	-	PAH/PSS	[117]

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Shell	9 μm	-	-	PLL, PR, DA, COL/HA, CS, DS, HS	[15]
Shell	3–6 μm	Protein	Insulin	PAH/PSS, PVS, DS	[20]
Shell	5 μm	Fluorescent dye	FITC-dextran	HA/PAH, PLL	[70]
Shell	3 μm	Fluorescent dye	FITC-dextran	pARG/DS, p(HPMA-DMAE)/PSS, PAH/PSS	[71]
Shell	~1 μm	Fluorescent dye, protein	Rhodamine B, methylene blue, insulin	Phenylboronic –modified alginate/PVPON	[94]
Shell	1.8–3.8 μm	-	-	pArg/DS	[118]
Shell	0.5 μm	Small molecule	Doxorubicin	pArg/DS	[32]
Shell	3–5 μm	Extract	*Gratiola officinalis* extract	PAH/PSS/DS	[97]
Shell	4 μm	Small molecule	Apigenin, ascorbic acid	PAH/DS	[96]
Shell	0.25–0.5 μm	Small molecule	Gemcitabine, clodronate	pArg/DS	[95]
Shell	3.3–4.8 μm	Protein	BSA, chymotrypsin, lysozyme	PAH/PSS	[103]
Shell	5.0–8.3 μm	Small molecule	Tetracycline hydrochloride	PAH/pectin	[99]
Shell	5.0 μm	Cells	*Escherichia coli*	PAH/PSS	[98]
Shell	4.5 μm	Small molecule	Doxorubicin, nimbin	PAH/PMA/NR	[119]
Shell	5.0 μm	Small molecule	Vitamin B12	PAH/PSS	[100]
Shell	5.0 μm	Protein	BSA	PAH/PSS	[105]
Shell	4.2–6.3 μm	-	-	PAH/PSS/QD	[36]
Shell	3–4 μm	Protein	Ovalbumin, horseradish peroxidase	pArg/DS	[104]
Shell	3 μm	Nucleic acid	G-quadruplex DNA, double stranded DNA	PMA/PVPON	[29]
Shell	0.65, 3.3 μm	Nucleic acid	mRNA, siRNA	pArg/DS	[107]
Shell	~3 μm	Nucleic acid	mRNA, pDNA, plasmid	pArg/DS/SiO_2	[106]
Shell	1–4 μm	Fluorescent dye	Tetramethylrhodamine dextran	PAH/DNA	[120]
Shell	2.84 μm	Labeled protein	BSA-Cy7	pArg/DS	[110]
Shell	3–5 μm	Small molecule	Doxorubicin	Chitosan/alginate	[69]
Shell	3–6 μm	Labeled protein	FITC-BSA	PLL/CS	[121]

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References

1. Bahrom H, Goncharenko AA, Fatkhutdinova LI, Peltek OO, Muslimov AR, Koval OY, et al. Controllable Synthesis of Calcium Carbonate with Different Geometry: Comprehensive Analysis of Particle Formation, Cellular Uptake, and Biocompatibility. ACS Sustainable Chemistry and Engineering. **2019**;7(23):19142-56.
2. Trushina DB, Bukreeva T V., Antipina MN. Size-Controlled Synthesis of Vaterite Calcium Carbonate by the Mixing Method: Aiming for Nanosized Particles. Crystal Growth and Design. **2016**;16(3):1311-9.
3. Vostrikova A V., Prikhozhdenko ES, Mayorova OA, Goryacheva IY, Tarakina N V., Sukhorukov GB, et al. Thermal carbonization in nanoscale reactors: Controlled formation of carbon nanodots inside porous CaCO3 microparticles. Scientific Reports. **2018**;8(1):1-7.
4. Trofimov AD, Ivanova AA, Zyuzin M V., Timin AS. Porous inorganic carriers based on silica, calcium carbonate and calcium phosphate for controlled/modulated drug delivery: Fresh outlook and future perspectives. Vol. 10, Pharmaceutics. **2018**.
5. Lam SF, Bishop KW, Mintz R, Fang L, Achilefu S. Calcium carbonate nanoparticles stimulate cancer cell reprogramming to suppress tumor growth and invasion in an organ-on-a-chip system. Scientific Reports. **2021**;11(1):1-12.
6. Ševčík R, Šašek P, Viani A. Physical and nanomechanical properties of the synthetic anhydrous crystalline CaCO3 polymorphs: vaterite, aragonite and calcite. Journal of Materials Science. **2018**;53(6):4022-33.
7. Svenskaya YI, Fattah H, Inozemtseva OA, Ivanova AG, Shtykov SN, Gorin DA, et al. Key Parameters for Size- and Shape-Controlled Synthesis of Vaterite Particles. Crystal Growth and Design. **2018**;18(1):331-7.
8. Han C, Hu Y, Wang K, Luo G. Preparation and in-situ surface modification of CaCO3 nanoparticles with calcium stearate in a microreaction system. Powder Technology. **2019**;356:414-22.
9. Niu YQ, Liu JH, Aymonier C, Fermani S, Kralj D, Falini G, et al. Calcium carbonate: controlled synthesis, surface functionalization, and nanostructured materials. Chemical Society Reviews. **2022**;51(18):7883-943.
10. Fadia P, Tyagi S, Bhagat S, Nair A, Panchal P, Dave H, et al. Calcium carbonate nano- and microparticles: synthesis methods and biological applications. 3 Biotech. **2021**;11(11):1-30.
11. Byrappa K, Ohara S, Adschiri T. Nanoparticles synthesis using supercritical fluid technology - towards biomedical applications. Advanced Drug Delivery Reviews. **2008**;60(3):299-327.
12. Pai RK, Pillai S. Nanoparticles of amorphous calcium carbonate by miniemulsion: Synthesis and mechanism. CrystEngComm. **2008**;10(7):865-72.
13. Anton N, Benoit JP, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates-A review. Journal of Controlled Release. **2008**;128(3):185-99.
14. Babou-Kammoe R, Hamoudi S, Larachi F, Belkacemi K. Synthesis of CaCO 3 nanoparticles by controlled precipitation of saturated carbonate and calcium nitrate aqueous solutions. The Canadian Journal of Chemical Engineering. 15 févr **2012**;90(1):26-33.
15. Campbell J, Abnett J, Kastania G, Volodkin D, Vikulina AS. Which biopolymers are better for the fabrication of multilayer capsules? A comparative study using vaterite CaCO3as templates. ACS Applied Materials and Interfaces. 2021;13(2):3259-69.
16. Chesneau C, Larue L, Belbekhouche S. Design of Tailor-Made Biopolymer-Based Capsules for Biological Application by Combining Porous Particles and Polysaccharide Assembly. Pharmaceutics. **2023**;15(6).
17. Tan C, Dima C, Huang M, Assadpour E, Wang J, Sun B, et al. Advanced CaCO3-derived delivery systems for bioactive compounds. Advances in Colloid and Interface Science. **2022**;309(September):102791.
18. Kalenichenko D, Nifontova G, Karaulov A, Sukhanova A, Nabiev I. Designing functionalized polyelectrolyte microcapsules for cancer treatment. Nanomaterials. **2021**;11(11):3055.
19. Li G, Zhao Y, Zhang J, Hao J, Xu D, Cao Y. CaCO3 loaded lipid microspheres prepared by the solid-in-oil-in-water emulsions technique with propylene glycol alginate and xanthan gum. Frontiers in Nutrition. **2022**;9(1).
20. Yoshida K, Ono T, Kashiwagi Y, Takahashi S, Sato K, Anzai JI. pH-dependent release of insulin from layer-by-layer-deposited polyelectrolyte microcapsules. Polymers. **2015**;7(7):1269-78.
21. Dou J, Zhao F, Fan W, Chen Z, Guo X. Preparation of non-spherical vaterite CaCO3 particles by flash nano precipitation technique for targeted and extended drug delivery. Journal of Drug Delivery Science and Technology. **2020**;57(April):101768.
22. Gundogdu D, Alemdar C, Turan C, Hazal Husnugil H, Banerjee S, Erel-Goktepe I. Tuning stimuli-responsive properties of alginate hydrogels through layer-by-layer functionalization for dual-responsive dual drug release. Colloids and Surfaces A: Physicochemical and Engineering Aspects. **2023**;676(PA):132213.
23. Decher G, Hong JD, Schmitt J. Buildup of ultrathin multilayer films by a self-assembly process: III. Consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces. Thin Solid Films. **1992**;210-211(PART 2):831-5.
24. Decher G, Hong JD. Buildup of ultrathin multilayer films by a self-assembly process, 1 consecutive adsorption of anionic and cationic bipolar amphiphiles on charged surfaces. Makromolekulare Chemie Macromolecular Symposia. 2 juin **1991**;46(1):321-7.

25. Vikulina AS, Campbell J. Biopolymer-Based Multilayer Capsules and Beads Made via Templating : Advantages , Hurdles and Perspectives. Nanomaterials. **2021**;11:2502.
26. Volodkin DV, Madaboosi N, Blacklock J, Skirtach AG, Möhwald H. Surface-Supported Multilayers Decorated with Bio-active Material Aimed at Light-Triggered Drug Delivery. Langmuir. 15 déc **2009**;25(24):14037-43.
27. De Geest BG, Skirtach AG, Mamedov AA, Antipov AA, Kotov NA, De Smedt SC, et al. Ultrasound-Triggered Release from Multilayered Capsules. Small. **2007**;3(5):804-8.
28. Déjugnat C, Sukhorukov GB. pH-Responsive Properties of Hollow Polyelectrolyte Microcapsules Templated on Various Cores. Langmuir. 1 août **2004**;20(17):7265-9.
29. Alford A, Tucker B, Kozlovskaya V, Chen J, Gupta N, Caviedes R, et al. Encapsulation and ultrasound-triggered release of G-Quadruplex DNA in multilayer hydrogel microcapsules. Polymers. **2018**;10(12).
30. Campbell J, Kastania G, Volodkin D. Encapsulation of Low-Molecular-Weight Drugs into Polymer Multilayer Capsules Templated on Vaterite CaCO 3 Crystals. micromachines. **2020**;11(717).
31. Li S, Lian B. Application of Calcium Carbonate as a Controlled Release Carrier for Therapeutic Drugs. Minerals. **2023**;13(9):1-11.
32. Gileva A, Trushina D, Yagolovich A, Gasparian M, Kurbanova L, Smirnov I, et al. Doxorubicin-Loaded Polyelectrolyte Multilayer Capsules Modified with Antitumor DR5-Specific TRAIL Variant for Targeted Drug Delivery to Tumor Cells. Nanomaterials. **2023**;13(5).
33. Fujiwara M, Shiokawa K, Araki M, Ashitaka N, Morigaki K, Kubota T, et al. Encapsulation of proteins into CaCO3 by phase transition from vaterite to calcite. Crystal Growth and Design. **2010**;10(9):4030-7.
34. Zhao D, Wang CQ, Zhuo RX, Cheng SX. Modification of nanostructured calcium carbonate for efficient gene delivery. Colloids and Surfaces B: Biointerfaces. **2014**;118:111-6.
35. Popova V, Poletaeva Y, Chubarov A, Dmitrienko E. pH-Responsible Doxorubicin-Loaded Fe3O4@CaCO3 Nanocomposites for Cancer Treatment. Pharmaceutics. 26 févr **2023**;15(3):771.
36. Nifontova G, Ramos-Gomes F, Baryshnikova M, Alves F, Nabiev I, Sukhanova A. Cancer cell targeting with functionalized quantum dot-encoded polyelectrolyte microcapsules. Frontiers in Chemistry. **2019**;7(JAN):1-11.
37. Trushina DB, Borodina TN, Belyakov S, Antipina MN. Calcium carbonate vaterite particles for drug delivery: Advances and challenges. Materials Today Advances. **2022**;14(2022):100214.
38. Ishikawa F, Murano M, Hiraishi M, Yamaguchi T, Tamai I, Tsuji A. Insoluble powder formulation as an effective nasal drug delivery system. Pharmaceutical Research. **2002**;19(8):1097-104.
39. Volodkin D V., Larionova NI, Sukhorukov GB. Protein encapsulation via porous CaCO3 microparticles templating. Biomacromolecules. **2004**;5(5):1962-72.
40. Parakhonskiy B V., Haase A, Antolini R. Sub-micrometer vaterite containers: Synthesis, substance loading, and release. Angewandte Chemie - International Edition. **2012**;51(5):1195-7.
41. Farzan M, Roth R, Québatte G, Schoelkopf J, Huwyler J, Puchkov M. Loading of porous functionalized calcium carbonate microparticles: Distribution analysis with focused ion beam electron microscopy and mercury porosimetry. Pharmaceutics. **2019**;11(1):1-14.
42. Levy CL, Matthews GP, Laudone GM, Beckett S, Turner A, Schoelkopf J, et al. Mechanism of adsorption of actives onto microporous functionalised calcium carbonate (FCC). Adsorption. **2017**;23(4):603-12.
43. Binevski P V., Balabushevich NG, Uvarova VI, Vikulina AS, Volodkin D. Bio-friendly encapsulation of superoxide dismutase into vaterite CaCO3 crystals. Enzyme activity, release mechanism, and perspectives for ophthalmology. Colloids and Surfaces B: Biointerfaces. **2019**;181(May):437-49.
44. Feoktistova NA, Balabushevich NG, Skirtach AG, Volodkin D, Vikulina AS. Inter-protein interactions govern protein loading into porous vaterite CaCO3crystals. Physical Chemistry Chemical Physics. **2020**;22(17):9713-22.
45. Li RG, Napoli E, Jorstad IS, Bønsdorff TB, Juzeniene A, Bruland ØS, et al. Calcium Carbonate Microparticles as Carriers of 224 Ra: Impact of Specific Activity in Mice with Intraperitoneal Ovarian Cancer. Current Radiopharmaceuticals. **2020**;14(2):145-53.
46. Westrøm S, Malenge M, Jorstad IS, Napoli E, Bruland ØS, Bønsdorff TB, et al. Ra-224 labeling of calcium carbonate microparticles for internal α-therapy: Preparation, stability, and biodistribution in mice. Journal of Labelled Compounds and Radiopharmaceuticals. 30 mai **2018**;61(6):472-86.
47. Feoktistova NA, Vikulina AS, Balabushevich NG, Skirtach AG, Volodkin D. Bioactivity of catalase loaded into vaterite CaCO3 crystals via adsorption and co-synthesis. Materials & Design. 5 janv 2020;185:108223.
48. Preisig D, Haid D, Varum FJO, Bravo R, Alles R, Huwyler J, et al. Drug loading into porous calcium carbonate microparticles by solvent evaporation. European Journal of Pharmaceutics and Biopharmaceutics. **2014**;87(3):548-58.
49. Roth R, Schoelkopf J, Huwyler J, Puchkov M. Functionalized calcium carbonate microparticles for the delivery of proteins. European Journal of Pharmaceutics and Biopharmaceutics. **2018**;122(October 2017):96-103.

50. Ramalapa B, Crasson O, Vandevenne M, Gibaud A, Garcion E, Cordonnier T, et al. Protein-polysaccharide complexes for enhanced protein delivery in hyaluronic acid templated calcium carbonate microparticles. Journal of Materials Chemistry B. **2017**;5(35):7360-8.
51. Vikulina AS, Feoktistova NA, Balabushevich NG, Skirtach AG, Volodkin D. The mechanism of catalase loading into porous vaterite CaCO3 crystals by co-synthesis. Physical Chemistry Chemical Physics. **2018**;20(13):8822-31.
52. Guo Y, Li H, Shi W, Zhang J, Feng J, Yang X, et al. Targeted delivery and pH-responsive release of doxorubicin to cancer cells using calcium carbonate/hyaluronate/glutamate mesoporous hollow spheres. Journal of Colloid and Interface Science. **2017**;502:59-66.
53. Feoktistova NA, Balabushevich NG, Skirtach AG, Volodkin D, Vikulina AS. Inter-protein interactions govern protein loading into porous vaterite CaCO3 crystals. Phys Chem Chem Phys. 6 mai **2020**;22(17):9713-22.
54. Wang P, Kankala RK, Fan J, Long R, Liu Y, Wang S. Poly-L-ornithine/fucoidan-coated calcium carbonate microparticles by layer-by-layer self-assembly technique for cancer theranostics. J Mater Sci: Mater Med. 10 mai **2018**;29(5):68.
55. 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. 15 mars **2017**;5(11):2068-73.
56. Guo Y, Li H, Shi W, Zhang J, Feng J, Yang X, et al. Targeted delivery and pH-responsive release of doxorubicin to cancer cells using calcium carbonate/hyaluronate/glutamate mesoporous hollow spheres. Journal of Colloid and Interface Science. 15 sept **2017**;502:59-66.
57. Muslimov AR, Antuganov D, Tarakanchikova YV, Karpov TE, Zhukov MV, Zyuzin MV, et al. An investigation of calcium carbonate core-shell particles for incorporation of 225Ac and sequester of daughter radionuclides: in vitro and in vivo studies. Journal of Controlled Release. 10 févr **2021**;330:726-37.
58. Lu J, Jiao Y, Cao G, Liu Z. Multimode CaCO3/pneumolysin antigen delivery systems for inducing efficient cellular immunity for anti-tumor immunotherapy. Chemical Engineering Journal. 15 sept **2021**;420:129746.
59. Wei Y, Sun R, Su H, Xu H, Zhang L, Huang D, et al. Synthesis and characterization of porous CaCO3 microspheres templated by yeast cells and the application as pH value-sensitive anticancer drug carrier. Colloids and Surfaces B: Biointerfaces. 1 mars **2021**;199:111545.
60. Ramalapa B, Crasson O, Vandevenne M, Gibaud A, Garcion E, Cordonnier T, et al. Protein–polysaccharide complexes for enhanced protein delivery in hyaluronic acid templated calcium carbonate microparticles. J Mater Chem B. 13 sept **2017**;5(35):7360-8.
61. Kudryavtseva VL, Zhao L, Tverdokhlebov SI, Sukhorukov GB. Fabrication of PLA/CaCO3 hybrid microparticles as carriers for water-soluble bioactive molecules. Colloids and Surfaces B: Biointerfaces. 1 sept **2017**;157:481-9.
62. Bewernitz MA, Lovett AC, Gower LB. Liquid–Solid Core-Shell Microcapsules of Calcium Carbonate Coated Emulsions and Liposomes. Applied Sciences. janv **2020**;10(23):8551.
63. Wei Y, Sun R, Su H, Xu H, Zhang L, Huang D, et al. Synthesis and characterization of porous CaCO3 microspheres templated by yeast cells and the application as pH value-sensitive anticancer drug carrier. Colloids and Surfaces B: Biointerfaces. **2021**;199(September 2020):111545.
64. Lybaert L, Ryu KA, Nuhn L, De Rycke R, De Wever O, Chon AC, et al. Cancer Cell Lysate Entrapment in CaCO3 Engineered with Polymeric TLR-Agonists: Immune-Modulating Microparticles in View of Personalized Antitumor Vaccination. Chemistry of Materials. **2017**;29(10):4209-17.
65. Kudryavtseva VL, Zhao L, Tverdokhlebov SI, Sukhorukov GB. Fabrication of PLA/CaCO3 hybrid microparticles as carriers for water-soluble bioactive molecules. Colloids and Surfaces B: Biointerfaces. **2017**;157:481-9.
66. Bewernitz MA, Lovett AC, Gower LB. Liquid–solid core-shell microcapsules of calcium carbonate coated emulsions and liposomes. Applied Sciences (Switzerland). **2020**;10(23):1-18.
67. Nifontova G, Tsoi T, Karaulov A, Nabiev I, Sukhanova A. Structure-function relationships in polymeric multilayer capsules designed for cancer drug delivery. Biomaterials Science. **2022**;10(18):5092-115.
68. Nifontova G, Zvaigzne M, Baryshnikova M, Korostylev E, Ramos-Gomes F, Alves F, et al. Next-Generation Theranostic Agents Based on Polyelectrolyte Microcapsules Encoded with Semiconductor Nanocrystals: Development and Functional Characterization. Nanoscale Research Letters. **2018**;13.
69. Zhao Q, Han B, Wang Z, Gao C, Peng C, Shen J. Hollow chitosan-alginate multilayer microcapsules as drug delivery vehicle: doxorubicin loading and in vitro and in vivo studies. Nanomedicine: Nanotechnology, Biology, and Medicine. **2007**;3(1):63-74.
70. Szarpak A, Cui D, Dubreuil F, De Geest BG, De Cock LJ, Picart C, et al. Designing hyaluronic acid-based layer-by-layer capsules as a carrier for intracellular drug delivery. Biomacromolecules. **2010**;11(3):713-20.
71. De Geest BG, Vandenbroucke RE, Guenther AM, Sukhorukov GB, Hennink WE, Sanders NN, et al. Intracellularly Degradable Polyelectrolyte Microcapsules. Advanced Materials. 18 avr **2006**;18(8):1005-9.

72. Ariga K, Lvov YM, Kawakami K, Ji Q, Hill JP. Layer-by-layer self-assembled shells for drug delivery. Advanced Drug Delivery Reviews. **2011**;63(9):762-71.
73. Prikhozhdenko ES, Gusliakova OI, Kulikov OA, Mayorova OA, Shushunova NA, Abdurashitov AS, et al. Target delivery of drug carriers in mice kidney glomeruli via renal artery. Balance between efficiency and safety. Journal of Controlled Release. 10 janv **2021**;329:175-90.
74. Zheng P, Ding B, Shi R, Jiang Z, Xu W, Li G, et al. A Multichannel Ca2+ Nanomodulator for Multilevel Mitochondrial Destruction-Mediated Cancer Therapy. Advanced Materials. **2021**;33(15):2007426.
75. Nifontova G, Ramos-Gomes F, Baryshnikova M, Alves F, Nabiev I, Sukhanova A. Cancer Cell Targeting With Functionalized Quantum Dot-Encoded Polyelectrolyte Microcapsules. Front Chem. 30 janv **2019**;7:34.
76. Flemke J, Maywald M, Sieber V. Encapsulation of Living E. coli Cells in Hollow Polymer Microspheres of Highly Defined Size. Biomacromolecules. 14 janv **2013**;14(1):207-14.
77. Gileva A, Trushina D, Yagolovich A, Gasparian M, Kurbanova L, Smirnov I, et al. Doxorubicin-Loaded Polyelectrolyte Multilayer Capsules Modified with Antitumor DR5-Specific TRAIL Variant for Targeted Drug Delivery to Tumor Cells. Nanomaterials. janv **2023**;13(5):902.
78. Gundogdu D, Alemdar C, Turan C, Husnugil HH, Banerjee S, Erel-Goktepe I. Tuning Stimuli-responsive Properties of Alginate Hydrogels through Layer-by-layer Functionalization for Dual-responsive Dual Drug Release. Colloids and Surfaces A: Physicochemical and Engineering Aspects. **2023**;132213.
79. Kalenichenko D, Nifontova G, Karaulov A, Sukhanova A, Nabiev I. Designing Functionalized Polyelectrolyte Microcapsules for Cancer Treatment. Nanomaterials. nov **2021**;11(11):3055.
80. Mihai M, Racovita S, Vasiliu AL, Doroftei F, Barbu-Mic C, Schwarz S, et al. Autotemplate Microcapsules of CaCO3/Pectin and Nonstoichiometric Complexes as Sustained Tetracycline Hydrochloride Delivery Carriers. ACS Appl Mater Interfaces. 25 oct **2017**;9(42):37264-78.
81. Navolokin N, Lomova M, Bucharskaya A, Godage O, Polukonova N, Shirokov A, et al. Antitumor Effects of Microencapsulated Gratiola officinalis Extract on Breast Carcinoma and Human Cervical Cancer Cells In Vitro. Materials. janv **2023**;16(4):1470.
82. Nehru S, Guru A, Pachaiappan R, Hatamleh AA, Al-Dosary MA, Arokiyaraj S, et al. Co-encapsulation and release of apigenin and ascorbic acid in polyelectrolyte multilayer capsules for targeted polycystic ovary syndrome. Int J Pharm. 28 déc **2023**;651:123749.
83. Novoselova MV, Loh HM, Trushina DB, Ketkar A, Abakumova TO, Zatsepin TS, et al. Biodegradable Polymeric Multilayer Capsules for Therapy of Lung Cancer. ACS Appl Mater Interfaces. 5 févr **2020**;12(5):5610-23.
84. Trushina DB, Bukreeva TV, Borodina TN, Belova DD, Belyakov S, Antipina MN. Heat-driven size reduction of biodegradable polyelectrolyte multilayer hollow capsules assembled on CaCO3 template. Colloids and Surfaces B: Biointerfaces. **2018**;170:312-21.
85. De Temmerman ML, Rejman J, Grooten J, De Beer T, Vervaet C, Demeester J, et al. Lyophilization of Protein-Loaded Polyelectrolyte Microcapsules. Pharm Res. 1 juill **2011**;28(7):1765-73.
86. Maiorova LA, Erokhina SI, Pisani M, Barucca G, Marcaccio M, Koifman OI, et al. Encapsulation of vitamin B12 into nanoengineered capsules and soft matter nanosystems for targeted delivery. Colloids and Surfaces B: Biointerfaces. 1 oct **2019**;182:110366.
87. Musin EV, Kim AL, Tikhonenko SA. Destruction of Polyelectrolyte Microcapsules Formed on CaCO3 Microparticles and the Release of a Protein Included by the Adsorption Method. Polymers (Basel). 1 mars **2020**;12(3):520.
88. Alford A, Tucker B, Kozlovskaya V, Chen J, Gupta N, Caviedes R, et al. Encapsulation and Ultrasound-Triggered Release of G-Quadruplex DNA in Multilayer Hydrogel Microcapsules. Polymers (Basel). 5 déc **2018**;10(12):1342.
89. Tarakanchikova YV, Muslimov AR, Zyuzin MV, Nazarenko I, Timin AS, Sukhorukov GB, et al. Layer-by-Layer-Assembled Capsule Size Affects the Efficiency of Packaging and Delivery of Different Genetic Cargo. Particle & Particle Systems Characterization. **2021**;38(2):2000228.
90. Timin AS, Muslimov AR, Lepik KV, Epifanovskaya OS, Shakirova AI, Mock U, et al. Efficient gene editing via non-viral delivery of CRISPR–Cas9 system using polymeric and hybrid microcarriers. Nanomedicine: Nanotechnology, Biology and Medicine. 1 janv **2018**;14(1):97-108.
91. De Geest BG, Vandenbroucke RE, Guenther AM, Sukhorukov GB, Hennink WE, Sanders NN, et al. Intracellularly Degradable Polyelectrolyte Microcapsules. Advanced Materials. **2006**;18(8):1005-9.
92. Yoshida K, Ono T, Kashiwagi Y, Takahashi S, Sato K, Anzai J ichi. pH-Dependent Release of Insulin from Layer-by-Layer-Deposited Polyelectrolyte Microcapsules. Polymers. juill **2015**;7(7):1269-78.
93. Szarpak A, Cui D, Dubreuil F, De Geest BG, De Cock LJ, Picart C, et al. Designing Hyaluronic Acid-Based Layer-by-Layer Capsules as a Carrier for Intracellular Drug Delivery. Biomacromolecules. 8 mars **2010**;11(3):713-20.
94. Belbekhouche S, Charaabi S, Carbonnier B. Glucose-sensitive capsules based on hydrogen-bonded (polyvinylpyrrolidone / phenylboronic –modified alginate) system. Colloids and Surfaces B: Biointerfaces. **2019**;177(January):416-24.

 doi:10.20944/preprints202404.0315.v1

95. Novoselova M V., Loh HM, Trushina DB, Ketkar A, Abakumova TO, Zatsepin TS, et al. Biodegradable Polymeric Multilayer Capsules for Therapy of Lung Cancer. ACS Applied Materials and Interfaces. **2020**;12(5):5610-23.
96. Nehru S, Guru A, Pachaiappan R, Hatamleh AA, Al-Dosary MA, Arokiyaraj S, et al. Co-encapsulation and release of apigenin and ascorbic acid in polyelectrolyte multilayer capsules for targeted polycystic ovary syndrome. International Journal of Pharmaceutics. **2024**;651(December 2023):123749.
97. Navolokin N, Lomova M, Bucharskaya A, Godage O, Polukonova N, Shirokov A, et al. Antitumor Effects of Microencapsulated Gratiola officinalis Extract on Breast Carcinoma and Human Cervical Cancer Cells In Vitro. Materials. **2023**;16(4).
98. Flemke J, Maywald M, Sieber V. Encapsulation of living E. coli cells in hollow polymer microspheres of highly defined size. Biomacromolecules. **2013**;14(1):207-14.
99. Mihai M, Racovita S, Vasiliu AL, Doroftei F, Barbu-Mic C, Schwarz S, et al. Autotemplate Microcapsules of CaCO3/Pectin and Nonstoichiometric Complexes as Sustained Tetracycline Hydrochloride Delivery Carriers. ACS Applied Materials and Interfaces. **2017**;9(42):37264-78.
100. Maiorova LA, Erokhina SI, Pisani M, Barucca G, Marcaccio M, Koifman OI, et al. Encapsulation of vitamin B12 into nanoengineered capsules and soft matter nanosystems for targeted delivery. Colloids and Surfaces B: Biointerfaces. **2019**;182(May).
101. Svenskaya Y, Garello F, Lengert E, Kozlova A, Verkhovskii R, Bitonto V, et al. Biodegradable polyelectrolyte/magnetite capsules for MR imaging and magnetic targeting of tumors. Nanotheranostics. **2021**;5(3):362-77.
102. Borbora A, Manna U. Impact of chemistry on the preparation and post-modification of multilayered hollow microcapsules. Chemical Communications. **2021**;57(17):2110-23.
103. Petrov AI, Volodkin D V., Sukhorukov GB. Protein-calcium carbonate coprecipitation: A tool for protein encapsulation. Biotechnology Progress. **2005**;21(3):918-25.
104. De Temmerman ML, Rejman J, Grooten J, De Beer T, Vervaet C, Demeester J, et al. Lyophilization of protein-loaded polyelectrolyte microcapsules. Pharmaceutical Research. **2011**;28(7):1765-73.
105. Musin E V., Kim AL, Tikhonenko SA. Destruction of polyelectrolyte microcapsules formed on CaCO3 microparticles and the release of a protein included by the adsorption method. Polymers. **2020**;12(3):1-8.
106. Timin AS, Muslimov AR, Lepik K V., Epifanovskaya OS, Shakirova AI, Mock U, et al. Efficient gene editing via non-viral delivery of CRISPR–Cas9 system using polymeric and hybrid microcarriers. Nanomedicine: Nanotechnology, Biology, and Medicine. **2018**;14(1):97-108.
107. Tarakanchikova Y V., Muslimov AR, Zyuzin M V., Nazarenko I, Timin AS, Sukhorukov GB, et al. Layer-by-Layer-Assembled Capsule Size Affects the Efficiency of Packaging and Delivery of Different Genetic Cargo. Particle and Particle Systems Characterization. **2021**;38(2):1-10.
108. Machtakova M, Thérien-Aubin H, Landfester K. Polymer nano-systems for the encapsulation and delivery of active biomacromolecular therapeutic agents. Chemical Society Reviews. **2022**;51(1):128-52.
109. 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(25):12639-47.
110. Prikhozhdenko ES, Gusliakova OI, Kulikov OA, Mayorova OA, Shushunova NA, Abdurashitov AS, et al. Target delivery of drug carriers in mice kidney glomeruli via renal artery. Balance between efficiency and safety. Journal of Controlled Release. **2021**;329(November 2020):175-90.
111. Muslimov AR, Antuganov D, Tarakanchikova Y V., Karpov TE, Zhukov M V., Zyuzin M V., et al. An investigation of calcium carbonate core-shell particles for incorporation of 225Ac and sequester of daughter radionuclides: in vitro and in vivo studies. Journal of Controlled Release. **2021**;330(September 2020):726-37.
112. d'Amora M, Liendo F, Deorsola FA, Bensaid S, Giordani S. Toxicological profile of calcium carbonate nanoparticles for industrial applications. Colloids and Surfaces B: Biointerfaces. **2020**;190(March):110947.
113. Lu J, Jiao Y, Cao G, Liu Z. Multimode CaCO3/pneumolysin antigen delivery systems for inducing efficient cellular immunity for anti-tumor immunotherapy. Chemical Engineering Journal. **2021**;420(P1):129746.
114. Lin J, Huang L, Xiang R, Ou H, Li X, Chen A, et al. Blood compatibility evaluations of CaCO3 particles. Biomedical Materials (Bristol). **2021**;16(5).
115. Wang P, Kankala RK, Fan J, Long R, Liu Y, Wang S. Poly-L-ornithine/fucoidan-coated calcium carbonate microparticles by layer-by-layer self-assembly technique for cancer theranostics. Journal of Materials Science: Materials in Medicine. **2018**;29(5).
116. 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. Journal of Materials Chemistry B. **2017**;5(11):2068-73.
117. Antipov AA, Shchukin D, Fedutik Y, Petrov AI, Sukhorukov GB, Möhwald H. Carbonate microparticles for hollow polyelectrolyte capsules fabrication. Colloids and Surfaces A: Physicochemical and Engineering Aspects. **2003**;224(1-3):175-83.

 doi:10.20944/preprints202404.0315.v1

118. Trushina DB, Bukreeva T V., Borodina TN, Belova DD, Belyakov S, Antipina MN. Heat-driven size reduction of biodegradable polyelectrolyte multilayer hollow capsules assembled on CaCO3 template. Colloids and Surfaces B: Biointerfaces. **2018**;170(March):312-21.
119. Sharma V, Vijay J, Ganesh MR, Sundaramurthy A. Multilayer capsules encapsulating nimbin and doxorubicin for cancer chemo-photothermal therapy. International Journal of Pharmaceutics. **2020**;582(April):119350.
120. Lin YH, Singuru MMR, Marpaung DSS, Liao WC, Chuang MC. Ethylene Glycol-Manipulated Syntheses of Calcium Carbonate Particles and DNA Capsules toward Efficient ATP-Responsive Cargo Release. ACS Applied Bio Materials. **2023**;6(8):3351-60.
121. Zhao Q, Li B. pH-controlled drug loading and release from biodegradable microcapsules. Nanomedicine: Nanotechnology, Biology, and Medicine. **2008**;4(4):302-10.