REVIEW ARTICLE

Potential of Calcium Carbonate Nanoparticles for Therapeutic Applications

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ABSTRACT

The application of nanoparticles (NPs) has attracted considerable attention as targeted delivery systems. $CaCO_3$ has become the focus due to its advantages including affordability, low toxicity, biocompatibility, cytocompatibility, pH sensitivity and sedate biodegradability and environment friendly materials. In this article, we will discuss the potential roles of $CaCO_3$-NPs in three major therapeutic applications; as antimicrobial, for drug delivery, and as gene delivery nanocarrier.

Keywords: Calcium carbonate nanoparticle, Antimicrobial agent, Drug delivery agent, Gene delivery agent, Nanomedicine

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INTRODUCTION

Nanotechnology has introduced new methods in producing of newly codified products with improved efficacies, namely; nanoparticles (NPs). NPs can be prepared from various materials such as metals, metal oxides, synthetic polymers, proteins, polysaccharides or other organic based molecules (1) with various applications ranging from as contrast agents in medical imaging technology to drug delivery nanosystems (2-4). $CaCO_3$ nanoparticles have attracted an interest among researchers nowadays especially for therapeutic applications. $CaCO_3$ present in three common polymorphs such as calcite, vaterite and aragonite. Calcite has high stability index and being studied in various sizes, shapes and structures (5, 6) and naturally found in trigonal crystalline form. The other two polymorphs exist in metastable forms are vaterite usually colourless with hexagonal crystal system (7) and aragonite is also naturally occurring carbonate minerals (8).

Calcium carbonate based material present the biodegradability and biocompatibility properties which is ideal as a smart carrier to deliver genes, enzymes, and drugs (9, 10). $CaCO_3$-NPs are also available in passivated, ultrahigh purity, high purity and coated and dispersed forms with dimensions ranging from 5–350 nm in diameter. Modification of $CaCO_3$-NPs with polymeric micelles has been investigated for thermodynamic and mechanical stability properties (11). Furthermore, modification of NPs for therapeutic applications have attracted considerable attention among researchers nowadays to improve the solubility, stability, circulation half-life and bio-distribution of the encapsulated agent. The potential roles of $CaCO_3$-NP for therapeutic application have been summarised in Fig. 1.

The pH-sensitive $CaCO_3$ nanoparticles are believed to be useful as a drug delivery system for diverse ailments, especially for anti-tumour agents. This is due to the fact that the increase in tumour growth is accompanied by approximately 5-times elevation in extracellular hydrogen ion concentration and a subsequent pH decrease (12). In the acidic medium of tumour tissue, $CaCO_3$ nanoparticles selectively accumulate and release its drug payload in a sustained fashion in addition to its ability to increase tumour extracellular pH. In this

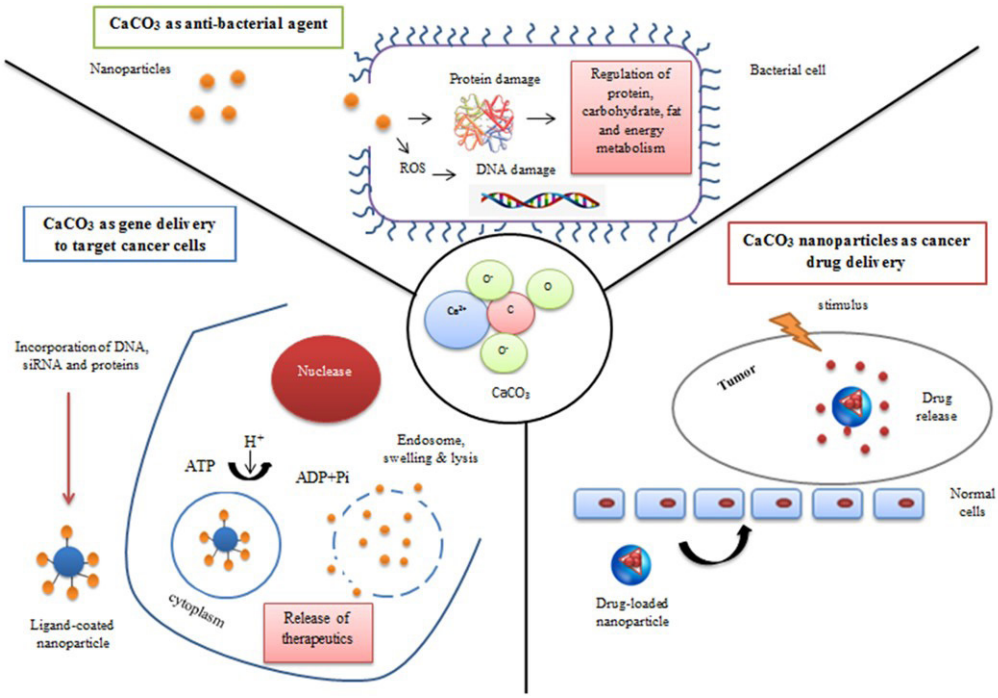

Figure 1: The Potential of $CaCO_3$-NPs for Therapeutics Applications. $CaCO_3$ nanoparticles have widely discussed as an agent for anti-microbial agent, drug and gene delivery agent in therapeutic purposes.

context, other strategies that were tested and compared with the efficacy of $CaCO_3$ nanoparticles to increase the extracellular pH of the tumour tissue in order to reduce its growth and metastasis. These strategies represented either by modulating intrinsic proton transport or by increasing the dietary sodium bicarbonate that in turn raises the systemic extracellular pH, but unfortunately this was faced by drug resistance due to multitude of intrinsic compensatory pathways, or the toxic effects caused by large amounts of salts ingested respectively (13).

The delivery system that consists of nanoparticles of any type is usually distinguished over microparticle system by that the particle small size of nano range (10 nm – 1000nm) can be administered by different routes such as intravenous, subcutaneous, intramuscular, transdermal or ophthalmic without causing mechanical entrapment in the capillaries as is the case with the microspheres. The microparticle size between 4 – 10 µm is usually trapped and filtered out when given intravenously and no beneficial effects can be observed, whereas the nanoparticles can pass blood brain barrier and evade reticuloendothelial system and finally accumulate in the site of action especially when their surface is modified with targeting agents or with specific surfactants (14).

$CaCO_3$ NANOPARTICLES SYNTHESIS

The classical synthesis pathways of $CaCO_3$-NP via reacting calcium chloride with sodium carbonate and sodium chloride using wet chemical precipitation technique is rather primitive and produces different crystal structures and morphologies in the large scale production. On the other hand, the mechanochemical processing using solid state chemical reactions produces uniform nanoparticles in size and morphology with low agglomeration rate and a mean particle size in the range of 4 nm (15). $CaCO_3$-NP can also be synthesized by sol-gel method that can produce uniform particle sizes with hydrophobic drugs incorporated into the nanoparticle matrix in the amorphous form. In this method, calcium ethoxide was used as a precursor and CO_2 sequestration synthesis to produce extremely small calcite $CaCO_3$ nanoparticles that form sols at the beginning followed by gels in the reaction media (16). Inverse micelle, exfoliation by polymerization, bimemtic synthesis and ultrasound cavitation technique are also used for synthesizing $CaCO_3$ nanoparticles. Furthermore, self-assembly of $CaCO_3$-NP can be achieved in water and hydrophobic solvents without surfactant to produce ellipsoidal spheres or with surfactants that gives spherical nanoparticles (17-19).

Polymer mediated growth (PMG) technique is also utilized to synthesize and control the size of $CaCO_3$ nanoparticles. The polymers usually used in the technique are polyethylene glycol, polyethylene oxide, polyacrylamide in addition to agarose gel. During the synthesis process, one ion of $CaCO_3$ is fixed on the polymer and the ambulatory ion of the other reactant comes from the external solution. This technique is simple and economic, can be performed under room temperature and the morphology of the nanoparticles and their particle size can be controlled. It noteworthy to mention that agarose gel is nontoxic and safer than acrylamide polymer, whereas the separation of the grown nanoparticles from the gel is easier than the sticky

PEG polymers (20).

$CaCO_3$ AS AN ANTIMICROBIAL AGENT

Researchers who are interested in developing antimicrobial agents and/or delivery system are currently shedding more light on nanoparticles as antimicrobials by itself or as antimicrobial delivery system. The inorganic NPs such as metal and metal oxides had effectively been used as antimicrobial agents due to their well-known highly potent antibacterial effect. The bactericidal properties of most metal oxide NPs are represented by ion (s) release and reactive oxygen species (ROS) generation mechanism. Several studies emphasised the different physicochemical effects of NPs in the typical size (9, 21) shape, types of chemical modification that greatly affect the antibacterial activities.

On the other hand, green nanomaterial such as $CaCO_3$-NPs could also provide an advantages in enhancing the intracellular penetration of antibiotics and retention time to achieve its efficacy thus it can be presented as potential antimicrobial agent delivery system. $CaCO_3$-NPs containing antibiotic could directly be phagocytosed by intracellular microbes host and sustain the release antibiotic against the intracellular microbes before developing resistance. Ataee et al. (21) showed that $CaCO_3$-NPs present as an excellent antibacterial agent against gram-negative and gram-positive bacteria such as Agrobacterium tumefaciens and Staphylococcus aureus. These findings have shown a promising application of $CaCO_3$-NPs as antimicrobial agents that may provide solutions regarding health issues related to microbial infections (21).

Bone infectious diseases such as osteomyelitis, are considered amongst the difficult infections for conventional treatment. Debridement of the surrounding tissue and amputation of the infected bone in addition to antimicrobial treatment with high serum concentration could be associated with high resistance levels and patient inconvenience. Decreasing treatment costs and side effects in addition to avoiding pain on patient are factors that push for creating nanocarrier that deliver antibiotic effecienctly and in directly targeted manner. Ciprofloxacin was loaded on $CaCO_3$-NPs prepared by W/O microemulsion method that achieved similar minimum inhibitory concentration (MIC) values as the free drug however, after 2 days of incubation, ciprofloxacin-loaded-$CaCO_3$ NP unlike the free drug solution showed antibacterial effects against Staphylococcus aureus which suggest the controlled-release effects of $CaCO_3$-NPs (22).

$CaCO_3$ AS DRUG NANOCARRIERS

Research over the years has contributed in developing different pharmaceutical products comprising nanoparticulate carriers such as polymeric and lipidic based NPs: liposomes, nanosuspension and nanoemulsions. NPs drug carriers can solve the present drug delivery issues by enhancing absorption properties, improving solubilisation, ameliorating formulation stability, and increasing shelf life which could result in better therapeutic efficiency and safety indices (23). For example, administration of drug coated NPs at systemic level might improve the circulation half-lives and pharmacokinetic activities thus reducing side effects (24). $CaCO_3$ also have been profiled in controlled drug release systems for long periods after administration (9). Various studies discussed $CaCO_3$-NPs as drug nanosystem from synthesis method to physiochemical characterisation and therapeutic application as shown in Table I.

Table I: Studies reported on the use of CaCO3-NPs as drug delivery carrier

Drug incorporated to $CaCO_3$ nanoparticles	Preparation method	Physiochemical characterization	Range of treatment	Incubation period	Major findings	Ref.
5-fluorouracil 5-FU-$CaCO_3$ nanoparticles	Manufactured $CaCO_3$= top-down ball-milling method	- porous, enabling drug loading - highly crystalline - calcite $CaCO_3$ - size ranging from ~10 to 60nm	- Target drug loading ~100µM for each batch of $CaCO_3$	-Gastric transit studies by using rabbit	By testing different tablet formulations, a cylindrical tablet provides the best radiographs in the rabbit model and proved to have an easier passage through the upper digestive tract.	(20)
Doxorubicin (DOX)-$CaCO_3$ nanoparticles	Manufactured $CaCO_3$= oil-in-water (O/W) microemulsions using higher pressure homogeniser (HPH)	- porous nature TEM: - perfect rod-shaped morphology - average size of 35nm to 60nm FESEM: - rod shape	- 0 to 2µg/ml DOX and $CaCO_3$/DOX	- incubate 24,48 and 72h	Bio-based calcium carbonate nanocrystal carrier effectively delivers a wide range of therapeutic drugs with pH-sensitive properties. The carrier has the capacity for large loads of anticancer drugs and is able to deliver these agents selectively to cancer cells with high specificity, achieving effective cancer cell death without inducing nonspecific toxicity. Slow release was observed at normal physiological pH (7.4) with a faster release at acidic pH (4.8) simulating tumour microenvironment. This study indicated that the DOX-loaded $CaCO_3$ nanocrystals are promising materials in the delivery of anticancer drugs.	(21)

$CaCO_3$ is pH-sensitive nanoparticles that enables the scientist to control its degradability rate depending on the target applications (25, 26). Efficient tumour tissue (pH < 6.5) and cell (pH = 4.5 to 5.5) targeting are achieved via the pH-sensitive behaviour of $CaCO_3$-NPs. Sustainable level of drug delivery is attained due to some important features, such as the slow degradability of $CaCO_3$-NPs and the specific targeting of cancer cells due to their potential function with targeting agents. In fact, designing functionalised $CaCO_3$ nanostructures opens a new perspective of delivery systems towards cancer cells. This combination produces targeted and efficient drug carrier for cancer diagnosis and therapy purposes whilst reducing the toxicity of anticancer drugs on healthy cells and tissues (27).

$CaCO_3$ AS A GENE DELIVERY AGENT

Delivery of therapeutic gene has become the most pivotal step in gene therapy. Thus delivering the therapeutic gene with an appropriate nanosystem have been widely studied (28, 29) for its efficiently and stably deliver the gene into targeted cells or organs without degrading and causing side effects (30, 31). Several gene delivery vectors from viral to nonviral approach have been developed to mediate gene transfections. Viral vectors are more potent than nonviral vectors; however, their limitations associated with toxicity concerns and construction problems (32). As a result of low immune response and safety, nonviral vectors have attracted increasing attention regardless of their low transfection efficiency. Green biomaterials have attracted attention to the nonviral vectors due to their unique properties, such as biodegradability, biocompatibility and controlled release (33).

$CaCO_3$ has excellent biocompatibility and low toxicity (28, 32, 34). $CaCO_3$-NPs could become a novel nonviral system for the effective delivery of small interfering RNA (siRNA) to be utilized in anticancer therapy. He et al. (35) have reported successful delivery of VEGF-C targeted siRNA-$CaCO_3$-NPs in in vitro and in vivo models. A significantly reduced VEGF-C expression was observed in SGC-7901 cells and further observation on subcutaneous xenografts model also showed dramatic suppression in the carcinogenesis. Numerous studies have reported on potential $CaCO_3$ as anticancer nanosystem including preparation method, physiochemical characterisation and major findings for some anticancer-loaded $CaCO_3$-NPs are summarised in Table II.

Table II: Studies reported on the use of CaCO3-NPs as gene delivery carrier

Gene incorporated to $CaCO_3$ nanoparticles	Cell line used	Preparation methods	Physicochemical characterization	Major findings	References
siRNA (siVEGF-C-$CaCO_3$ nanoparticles)	SGC-7901 (gastric cancer cell line)	Microemulsion method	PS*: 58 nm ZP*: +28.6 mV TE*: 65%	-High transfection efficiency with both nanoparticle (approximately 65%) and lipofection approaches (approximately 70%). -Efficient as vector for DNA transfection	(27)
siRNA ($CaCO_3$/$CaIP_6$ nanoparticle-siAKT1)	MCF-7 (human breast tumor cells)	No mention	Morphology: spherical particles PS: 80-200 nm Composition: approximately 90% ACC and 10% $CaIP_6$ ZP: +30.81 mV	-The percentage of fluorescent cells (successfully transfected cells) increased with higher FAM-siRNA concentrations. -Optimal siRNA concentration for ACC/CaIP6 nanoparticle delivery in MCF-7 cells was 150 nm at a mass ratio of 50:1.	(22)
DNA Plasmid (DNA–ACC/CaIP6 complexes)	Human vascular smooth muscle cells (HVSMC)	Chemical precipitation: Ethanol and double distilled water as binary solvent reaction system	Morphology: spherical PS: 80–200 nm ZP: +30.81 mV	-Functional ACC/CaIP6 nanocomposite particles display higher transfection efficiency (50%) than commercial Lipofectamine 2000 (35%). -ACC/CaIP6 nanocomposite particles exhibited much higher level of cell viability (92%) at a concentration of 100 mg mL, close to the DNA only sample.	(26)
pEGFP-C1-p53-gene-loaded PEI-CaCO3	Hep3B, QSG-7701, H1299, 293a and Hela cells (Human cell lines)	Chemical precipitation: ethanol and distilled water as binary solvent reaction system	Morphology: spherical PS: 900 nm	-The gene expression of the $CaCO_3$ based approach is strongly affected by the Ca^{2+}/CO_3^{2-} ratio because the size of $CaCO_3$/DNA co-precipitates is mainly determined by the Ca^{2+}/CO_3^{2-} ratio. -The encapsulation efficiency of DNA increases with decreasing Ca^{2+}/CO_3^{2-} ratio.	(20)

*PS, ZP and TE stand for particle size, zeta potential and transfection efficiency, respectively.

Furthermore, the biocompatibility and biodegradability of $CaCO_3$-sNPs as gene delivery agent could be achieved by Ca2+ co-precipitation that can form ionic complexes with the nucleic acid backbone and ultimately stabilises the DNA structures (9). Moreover, this modification enables $CaCO_3$ NPs–DNA complexes effectively transported via ion channel-mediated endocytosis to cross the cell membranes (36). On the other hand, $CaCO_3$-NPs transport through endosome and osmotic imbalance are also possible by modulating pH media due the pH sensitivity of these nanoparticles.

FUTURE TRENDS

Nanosized calcium carbonate can replace the microsized calcium carbonate because it achieves higher bioavailability therefore it can be used in osteoporotic patients via oral administration with higher levels of convenience and less side effects (37). In the context of antimicrobial delivery system, $CaCO_3$-NPs can be utilized for infections that needs higher serum level concentrations in tissues that need specific penetration to deliver the antibiotic required. At the same time, it can replace local antibiotic delivery systems such as implantable antibiotic pump and cements because it needs no replacement or refills and provides higher convenience level for patients thanks to its controlled-release property characterized by $CaCO_3$-NP adsorption on bacterial cell wall (22). The pH-sensitive $CaCO_3$ NPs provides an insight for future treatments for solid cancerous diseases due to their acidic microenvironment that attract the $CaCO_3$ NPs specifically to unload its antitumor drug in a controlled-release way because of nanoparticles slow biodegradability (27). New future directions for preparing drug-loaded $CaCO_3$ NPs that either depends on chemical precipitation with modifications such as sol-gel technique that discussed earlier in this article, or on newly-introduced emulsion techniques such as ultrasound cavitation method.

CONCLUSION

In conclusion, $CaCO_3$-NPs could have potential roles in future therapeutic applications due to bio-accessibility, bio-availability and it is economically affordable. Their role in bone scaffolding, tissue engineering and gene and drug delivery is important and could replace many old techniques and treatment methods in diseases such as cancer microbial infections. Further studies are needed to contribute knowledge in field.

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