





Inorganic nanocarriers based on calcium carbonate and silica oxide for passive breast cancer targeting

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Abstract

Nanoparticles (NPs) are widely used platforms for delivery of various biologically active compounds. Unfortunately, there is a lack of comprehensive investigations that would include a few types of NPs with different physicochemical parameters and their potential use as delivery systems in one tumor model. Therefore, to achieve therapeutic effect via nanocarrier with therapeutic agent, the properties of the developed NPs must be clearly defined. Herein, we report the development and modification of ^{99m}Tc and Cy5-labeled NPs based on calcium carbonate (CaCO₃) and silica oxide (SiO₂) to investigate *in vitro* and *in vivo* distribution on an example of a breast cancer model. We describe the synthesis and characterization of these NPs, including their morphology, size distribution, stability in biological media and cytotoxicity. Transmission electron microscopy (TEM), confocal laser scanning microscopy (CLSM), dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), direct radiometry analysis, and histology were used to collect all data. The cellular uptake of NPs on 4T1 cell line was shown *in vitro* and *in vivo*. As a result, we demonstrated that these NPs are non-toxic, biocompatible, and stable system to use for delivery of bioactive compounds into breast cancer.

Keywords

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Key findings

- CaCO₃ and SiO₂ NPs can be a platform for delivery to breast cancer.
- Controlling the size and structure of CaCO₃ and SiO₂ NPs allows maintaining their efficient accumulation in a tumor.
- CaCO₃ and SiO₂ NPs accumulated in the breast cancer tumor with 1.62% ± 0.3% and 2.48% ± 0.4% ID/g, respectively.

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1. Introduction

Application of various types of inorganic nanoparticles (NPs) is of particular interest for delivery of biologically active, against malignant tumors, compounds [1–3]. Food and Drug Administration (FDA) approved biomaterials are required for the synthesis of NPs. Due to their advantages,

like stability, controllable surface modification and drug release, NPs are ideal candidates for the clinical applications. Among plenty of investigated biocompatible materials used for synthesis of NPs, calcium carbonate (CaCO₃) and silica oxide (SiO₂) draw the most attention. Ca²⁺ is the element that is present in human body. Due to the

biocompatibility, high loading capacity and porosity suitable for therapeutic molecule accommodation, CaCO_3 repossesses remarkable advantages in drug delivery systems [4]. Moreover, it was noted that CaCO_3 NPs can be effectively loaded with various bioactive components to treat malignant tumors [5, 6]. CaCO_3 NPs can be obtained with a chemical co-precipitation method. The main issue of co-precipitation method used for synthesis of CaCO_3 NPs is controlling the size distribution [7]. CaCO_3 NPs obtained with this technique are non-toxic and biocompatible [8]. Meanwhile, SiO_2 is a widely used biomaterial in nanomedicine. Due to high excellent adsorption capacity and chemical stability, SiO_2 is widely used for the synthesis of NPs [9, 10]. Sol-gel method [11] is one of the best methods for the preparation of SiO_2 NPs. In particular, this technique is extensively used because of easy methodology, soft conditions, and possibility for controlling the size of obtained NPs [12]. In fact, for biodistribution investigation and accumulation efficacy in tumor site, NPs parameters such as size and charge should be comparable. Thus, the NPs with similar parameters could be obtained via modified Stöber sol-gel method. In addition, different organic additives can be used for surface modification of NPs with precise shapes and structures. In this work, we adjusted the synthetic parameters (salt concentrations, process temperature, stirring time and velocity) to develop SiO_2 NPs using sol-gel method and CaCO_3 NPs using co-precipitation technic in the presence of polyacrylic acid (PAA) as an organic additive to improve colloidal stability of these NPs [13].

The surface modification of described NPs allows to perform conjugation with diagnostic isotope and fluorescent dyes for *in vitro* and *in vivo* NPs visualization [14,15]. According to the previous studies, we can highlight appropriate variants of diagnostic agents for modification of NPs, which include 99m-technetium ($^{99\text{m}}\text{Tc}$) and Cyanine 5 [16]. Thus, these diagnostic agents were used for visualization to detect the biodistribution of developed NPs. Despite of the great potential of described NPs, there is a lack of information about their biodistribution and tumor accumulation of functionalized NPs with one particular cancer model. In fact, it prevents introducing CaCO_3 and SiO_2 NPs as nanocarriers for delivery of therapeutic molecules to treat malignant tumor.

The limitations of conventional therapy induce the treatment paradigm shift toward the use of tumor-specific targeting, which may improve therapeutic efficacy. New combined therapies with the use of NPs are more promising than monotherapies. For instance, combined chemo-radiotherapy with the use of SiO_2 NPs leads to two times higher efficiency compared to conventional therapy [17]. Nevertheless, there is still quite a lack of information in this field.

Due to the effect of enhanced permeability and retention (EPR) through specific passive targeting, the side effects associated with the chemotherapeutic agent are ultimately reduced, and the antitumor effect is enhanced. Passive, nonspecific targeting is driven by NPs, reduced sizes

and unique properties, such as surface modifications, surface charge or nonspecific adhesion, which can lead to reaching organs with porous endothelial capillaries (liver, spleen), facilitating the crossing of specialized epithelium by NPs and penetration into the cell cytoplasm [18]. For example, in the case of cancer, the phenomenon of EPR is observed due to the specific tumor microenvironment, intense angiogenesis, uncontrollable growth of capillary net, etc.

The physicochemical properties of NPs have a significant impact on their biocompatibility and biodistribution. In order to address the biosafety of NPs, it is necessary to perform full characterization [19]. The characteristic feature of NPs, such as size, shape, size distribution, surface area, shape, solubility, aggregation, etc., need to be evaluated before assessing toxicity or biocompatibility. To do this many analytical techniques have been used, including Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), and so on [20]. Thus, it is very important to compare physicochemical properties of CaCO_3 and SiO_2 NPs. There is a lack of comparative knowledge of delivery efficacy of CaCO_3 and SiO_2 NPs towards breast cancer.

2. Materials and methods

2.1. Materials

Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $M_w = 147.01$), anhydrous sodium carbonate (Na_2CO_3 , $M_w = 105.99$) and polyacrylic acid (PAA, No. 32667) were obtained from Sigma-Aldrich (Germany). Tetraethylorthosilicate (TEOS, 99%) and ethanol (EtOH, 99.9%) were purchased from Merck (Germany). Purified water with a specific resistivity higher than $18.2 \text{ M}\Omega \text{ cm}^{-1}$ from a three-stage Milli-Q Plus 185 purification system was used. Cyanine5 (Cy5) dye, obtained from Lumiprobe Life Science solutions (Cockeysville, MD, USA), Alexa Fluor 488 (Thermo Fisher Scientific) and Propidium Iodide (PI) (Molecular Probes, Eugene, OR) were used for CLSM cellular visualization. Bovine serum albumin (BSA, 66 kDa) was bought in CSL Behring (Switzerland), dimethyl sulfoxide (DMSO) was purchased from Sigma chemical company. For $^{99\text{m}}\text{Tc}$ radiolabeling of NPs Tin(II) chloride dihydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, 98%) was purchased from Merck (Germany), hydrochloric acid (>35%, TraceSELECT) was purchased from Honeywell-Fluka (USA). Sodium hydroxide (BioXtra, $\geq 98\%$) and TWEEN® 80 were purchased from Sigma-Aldrich (Germany). The 4T1 cells were maintained in RPMI 1640 medium containing 10% of fetal bovine serum (FBS) (HyClone, USA).

2.2. Preparation of NPs

2.2.1. CaCO_3 NPs

A co-precipitation reaction was used to obtain polymer functionalized CaCO_3 NPs [8, 21]. For that, 1 mL of CaCl_2 aqueous solution (0.1 M) was added to 1 mL of PAA aqueous

solution (3.0 mg/mL) and stirred at room temperature for 1 h. Then, 1 mL of Na₂CO₃ aqueous solution (0.1 M) was added to the suspension. The final mixture was gently stirred for 1 h under the same conditions. Then, the suspension was washed multiple times with ethanol and purified by centrifugation for 3 min at 13,000 rpm after the reaction. Finally, the pellets were obtained and stored for further investigations.

2.2.2. SiO₂ NPs

The synthesis of SiO₂ NPs was performed using the modified sol-gel method [22]. First, 8.75 mL of 99% ethanol was mixed with 2.40 mL of Milli-Q water. After 5 min, 65 µL of 99% tetraethylorthosilicate (TEOS) and 390 µL of ammonia aqueous solution (NH₃·H₂O, 37%) were added to the solution drop by drop. The final mixture was vigorously stirred for 2 h. Next, the entire contents were poured into 2 mL tubes (Eppendorf, Germany) and centrifuged for 5 min at 12000 rpm. The formed SiO₂ NPs were washed by centrifugation at 12000 rpm for 5 min. The washing step was repeated four times: once with 99% ethanol and three times with Milli-Q water.

2.3. Radio- and fluorescent NPs functionalization reparations

2.3.1. Radiolabelling with ^{99m}Tc

The CaCO₃ and SiO₂ NPs were obtained as described above. Then, 300 µL of SnCl₂ (7 mg/mL, 2% HCl solution) and 1 µL of Tween 80 were added to each pellet with NPs (200 µg). The suspension was carefully resuspended, and 100 µL of ^{99m}TcO₄⁻ (solution in 0.9% NaCl) was added to the suspension of NPs. The final suspension of NPs was diluted up to 1 mL using 0.9% NaCl solution. The samples were incubated in a thermomixer for 1 h (1000 rpm, 25 °C). Then, the pH of the reaction was adjusted to pH 6.5–7.0 with 1 M NaOH. The radiolabeled NPs were washed 3 times (12,000 rpm, 5 min) and resuspended in 1 mL of 0.9% NaCl solution. The radiolabeling procedure was presented in previous work [16].

2.3.2. Fluorescent labelling with Cy5 de

2.3.2.1. Formation of Cy5-BSA complex

BSA was fluorescently labeled with Cy5 using the method described previously [23]. For that, 144 mg of BSA was added to 40 mL of PBS (pH 7.4) to a final concentration of 3.85 mg/mL. Further, 10 µg of Cy5 was resuspended in 4.4 mL of DMSO. Next, the dye mixture was combined with BSA and incubated for 24 h at 4 °C. Then, the unbound dye was removed via dialysis procedure [24]. For this, the resulting solution was poured into a dialysis bag, which was then placed in a beaker and stirred for 3 d (500 rpm, 4 °C). The water with non-reacted dye was changed every 24 h.

2.3.2.2 Cy5-labeling of CaCO₃ NPs

To obtain Cy5-labeled CaCO₃ NPs, 1 mL of 0.1 M CaCl₂ aqueous solution was added separately to 500 µL of the Cy5-BSA

complex and then stirred at room temperature for 1 h. After that, 1 mL of PAA aqueous solution (3.0 mg/mL) was added, and stirring was continued for 1 h. Next, 1 mL of Na₂CO₃ aqueous solution (0.1 M) was added to the resulting mixture and left for 1 h. The resulting solution was washed multiple times with ethanol and purified by centrifugation for 3 min at 13000 rpm after the reaction until there was no free dye left. Finally, the solvent was exchanged to PBS for further use.

2.3.2.3 Cy5-labeling of SiO₂ NPs

To obtain Cy5-labeled SiO₂ NPs, 500 µL of Cy5-BSA (5 mg/mL) was added to NPs during the sol-gel synthesis using the co-precipitation method. Finally, the SiO₂ NPs with Cy5 were washed twice (12 000 rpm, 3 min) with Milli-Q water to remove the unbound dye.

2.4. Characterization of NPs

2.4.1. Transmission electron microscopy

The shape and size of the obtained NPs were analyzed using transmission electron microscope (TEM) with a JEOL JEM-1400 model (JEOL LTD, Tokyo, Japan) operated at the voltage of 100 kV.

2.4.2. DLS and zeta potential

The hydrodynamic diameter (D_h) of the NPs was measured using BK Winner803 DLS Nano Particle Size Analyzer. The zeta potential of the CaCO₃ and SiO₂ NPs was determined in phosphate buffered solution (PBS) with pH 7.4 using ZetaSizer Ultra device (Malvern Instruments, UK). Three measurements were made for each sample.

2.4.3. FTIR

Fourier transform infrared spectroscopy (Tensor 27, Bruker, Germany) was used to characterize the functional groups of CaCO₃ and SiO₂ NPs. For each spectrum, 50 scans between the wavenumbers of 4000 and 400 cm⁻¹ were recorded in an attenuated total reflection mode.

2.4.4. Cellular uptake studies

To visualize cellular uptake of the NPs, the 4T1 cells were seeded into 24-well plate (1.0·10⁴ per well). After incubation, the Cy5-fluorescent labeled NPs were added to the cells at different concentrations (0.5 mg/mL, 1.0 mg/mL, and 2.0 mg/mL), and the cells were left overnight. Next day, the cell cytoskeletons were stained with phalloidin conjugated with AlexaFluor 488 (AF488), and the cell nuclei were stained blue with Propidium Iodide (PI). Cellular uptake of the NPs was visualized using a confocal laser scanning microscope (CLSM), Leica TCS SP8 (Germany). To visualize the cell cytoskeletons and cell nuclei, an argon laser emitting at 488 nm and a helium-neon laser emitting at 543 nm were used, respectively. To visualize Cy5-labeled NPs, a helium-neon laser emitting at 633 nm was used. The confocal pinhole was set to 1 Airy unit, and the images were taken with an HC PL FLUOTAR 10x/0.30 PH2 Objective.

2.4.5. Tumor model formation

For *in vivo* studies, healthy, immunocompetent Balb/c mice (males, eight weeks old, 18–22 g) from the St. Petersburg center of laboratory animals “Rappolovo” of the national research center “Kurchatov Institute” and were used. The mice were housed in cages under controlled environmental conditions, with access to food and water provided *ad libitum*, and were quarantined in a specific pathogen-free environment.

To establish the 4T1 metastatic tumor model, male Balb/c mice (eight weeks old, 18–22 g) were intravenously injected with 4T1 cells (0.1 mL, 10^6 cells/mL) through the tail vein. On the 10th d after the infusion of tumor cells, the mice were randomly divided into experimental groups, and the therapy was started. All the animal experiment protocols were approved by the veterinary office of the Russian Research Center for Radiology and Surgical Technologies and its local ethics committee.

2.4.6. Radiometry/dosimetry of *ex vivo* main organs and tumours

Direct radiometry analysis of the biodistribution of the ^{99m}Tc -labeled NPs in the tumor-bearing Balb/c mice was performed using a TRIATHLER portable spectrometric radiometer (Hidex Oy, Finland). Each animal was injected intravenously with ^{99m}Tc -labeled CaCO_3 and SiO_2 NPs in the same dose (12 MBq, 100 μL , 2 mg/mL). The animals were sacrificed. Then, the main organs (the heart, lungs, liver, spleen, kidneys) and tumors were removed from the body and weighed. Total radioactivity for each tested sample was calculated as the percentage of the injected activity adjusted by the weight of tissues (% dose/g).

2.4.7. Fluorescent histology of tumour tissue

Histological tissue sections were obtained from euthanized mice 24 h after the injection of Cy5-labeled CaCO_3 and SiO_2 NPs. The biopsy samples were isolated from the tumors and cut into 2–4 μm thick sections using a HM 340E rotary microtome (Thermo Scientific). Next, the samples were placed in a solution of PI (10 mg/mL) for 5 min to fluorescently stain the cell nuclei. After that, the sections were washed in PBS solution and enclosed in a glycergel. Then, the fixed samples were visualized under CLSM (with an objective EC PlanNeofluar 40x/1.30 Oil DIC).

3. Results and Discussion

3.1. Preparation

3.1.1. Preparation of CaCO_3 NPs

The synthesis of polymer functionalized CaCO_3 NPs was carried out using a co-precipitation reaction between aqueous solutions of CaCl_2 and Na_2CO_3 in the presence of PAA (Figure 1A). The PAA is an anionic polymer (negatively charged) containing carboxylic groups with relative electrostatic interaction with calcium ions, which influence the morphology and crystal growth of CaCO_3 . Additional surface modification affects the aggregation

and crystallization behavior of CaCO_3 NPs. TEM images showed that the NPs changed their morphology from irregular shape to spherical shape when the concentration of PAA was increased (Figure 2a). The CaCO_3 NPs were not universally spherical, and their surface was slightly rough. Some NPs were observed to be interconnected with each other. In fact, PAA was negatively charged and could interact with calcium ions to aggregate around it. Thus, increasing the PAA concentration influences the morphology of CaCO_3 NPs and promotes their spherical form.

3.1.2. Preparation of SiO_2 NPs

SiO_2 NPs were synthesized using the previously described sol-gel method [25], which involves the hydrolysis reaction of TEOS in water and ethanol in the presence of ammonium hydroxide solution with forming $\text{Si}(\text{OH})_4$. The scheme of synthesis is presented in Figure 2b. The hydrolysis reaction of TEOS proceeds through the formation of reactive Si–OH species and subsequent condensation of bonds leading to the formation of the Si–O–Si bridges [24, 26]. The condensation of the supersaturated silicic acid was indicated by an increasing opalescence of the mixture starting 2–10 min after adding the TEOS [25].

3.2. Characterization

3.2.1. Size, morphology, TEM and DLS characterization

The optimal parameters were selected to obtain CaCO_3 NPs and SiO_2 NPs. TEM revealed CaCO_3 NPs and SiO_2 NPs with the size distribution in the range of 50–120 nm (Figure 2a). NPs were described as spherical with slightly rough surface for SiO_2 NPs and scalenohedral for CaCO_3 NPs. Meanwhile, the size distribution of CaCO_3 and SiO_2 NPs was estimated by measuring the hydrodynamic diameter (D_h) values in an aqueous solution. The average D_h values according to the DLS analysis were 70–120 and 100–150 nm for modified CaCO_3 and SiO_2 NPs, respectively (Figure 2b). Electrochemical surface features of modified NPs were observed through zeta potential measurements. The zeta potentials in deionized water of modified SiO_2 and CaCO_3 were -0.96 and -10 mV, respectively. The delivery efficacy of NPs with neutral zeta potential or one in the range of $-10/+10$ mV was reported to be higher than that of NPs with positive (> 10 mV) or negative (< -10 mV) zeta potential [27].

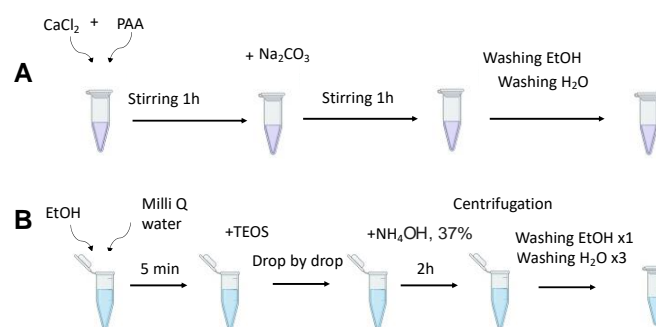


Figure 1 Scheme of CaCO_3 NPs synthesis (a). Scheme of SiO_2 NPs synthesis (b).

The less negatively charged CaCO_3 and SiO_2 NPs were chosen for biodistribution estimation *in vivo* [28–30]. Tumor accumulation was higher with zeta potential < -10 mV. Thus, appropriate zeta potentials of NPs increase their delivery efficacy.

PAA modified CaCO_3 NPs did not express any significant aggregation compared to NPs obtained without PAA coating (Figure 3a). At the same time, modified SiO_2 showed spherical morphology (Figure 3b). The presence of SnCl_2 used for radiolabeling of SiO_2 NPs did not influence on the morphology of the final products.

To further study the structural characteristics of developed NPs, FTIR spectra measurements of CaCO_3 and SiO_2 NPs were performed (Figure 4). According to Figure 4a, the FTIR spectrum of CaCO_3 NPs is characterized by the presence of stretching vibrations of the C–O approximately at 1412 cm^{-1} and bending vibrations of the C–O approximately at 862 and 683 cm^{-1} [31]. Meanwhile, the FTIR spectrum of SiO_2 NPs displays three characteristic absorption peaks approximately at 1070 , 950 and 800 cm^{-1} that shows the Si–O–Si stretching vibration bonding (Figure 4b). Absence of signals in the range from 3000 to 4000 cm^{-1} indicated the absence of H_2O molecules (in this range, vibrations of O–H bonds of water molecules appear) [32].

3.2.2. Cell uptake and distribution assay

The cellular uptake study was performed to reveal the internalization and association of CaCO_3 and SiO_2 NPs with breast cancer cells (4T1). For this, CLSM imaging and flow cytometry analysis were used. The cell nucleus was stained with PI and the actin cytoskeleton was labeled with Alexa Flour 488 Phalloidin. Meanwhile, Cy5-labeled CaCO_3 and SiO_2 NPs were used. The Cy5-labeled NPs (2 mg/mL) were incubated with cells for 24 h.

CLSM displayed significant uptake and distribution of Cy5-labeled NPs within 4T1 cells (Figure 5a). The quantitative data of cell association was $\sim 56.5\%$ for CaCO_3 NPs and $\sim 64.7\%$ for SiO_2 NPs (Figure 5b). The cell viability was $> 83\%$ for wide concentrations of NPs (Figure 5c). These observations demonstrated that two types of Cy5-labeled NPs have high cellular uptake in the 4T1 tumor.

The quantitative assessment of biodistribution of the $^{99\text{m}}\text{Tc}$ -labeled NPs was evaluated with direct radiometry analysis. After 24 h, the mice were euthanized, and the organs (the heart, lungs, liver, spleen, kidneys) and tumors were collected for the analysis. The obtained radioactivity data are presented in Table 1 in dose % per gram values (% dose/g).

According to Table 1, the $^{99\text{m}}\text{Tc}$ -labeled NPs were mainly accumulated in the liver (~ 82 – 84%) and only small amounts of NPs were detected in the tumor ($< 3\%$). The radioactivity in the 4T1 tumors was higher in $^{99\text{m}}\text{Tc}$ -labeled SiO_2 NPs than in $^{99\text{m}}\text{Tc}$ -labeled CaCO_3 NPs: $2.48\% \pm 0.4\%$ and $1.62\% \pm 0.3\%$. The higher percentage of $^{99\text{m}}\text{Tc}$ -labeled

NPs accumulation was detected in the liver due to mononuclear phagocyte system, which agrees with the literature data [18]. The NPs can accumulate in the liver due to their size, shape, surface coating, and the animal model [32]. The exception is NPs smaller than 6 nm, which can be filtered by kidneys and excreted into the urine [33].

3.2.3. Histological analysis

The fluorescent histological analysis demonstrated the distribution of Cy5-labeled CaCO_3 and SiO_2 NPs within tumor 4T1 tissues (Figure 6). The results of fluorescent histological analysis revealed the same trend in accumulation of NPs as it was observed in case of radiometry analysis. It could be explained by tumor model heterogeneity, variety of blood vessels and network of microcapillaries, supplying tumor with nutrients and oxygen [34, 35].

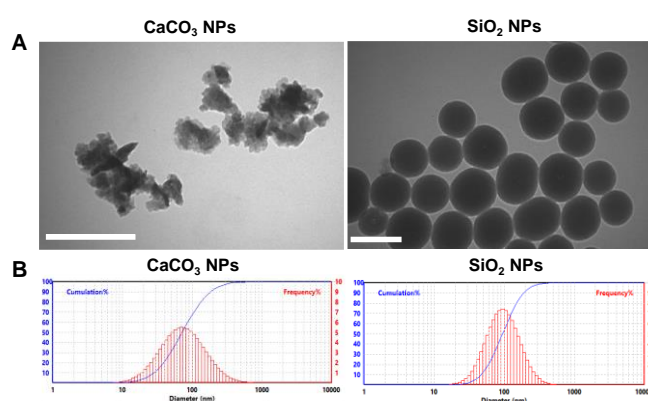


Figure 2 TEM images of CaCO_3 NPs (left) and SiO_2 NPs (right) (a). Scale bar = 200 nm. DLS analysis of NPs hydrodynamic diameters (D_h) (b).

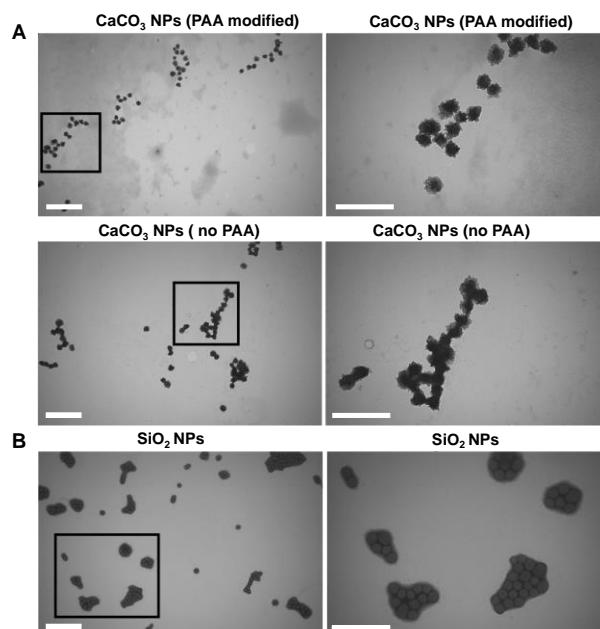


Figure 3 TEM images of CaCO_3 NPs (PAA/no PAA modification) (a). TEM images of SiO_2 NPs modifications with SnCl_2 modified for $^{99\text{m}}\text{Tc}$ -labeling. Scale bar = 1000 nm (left column) and 500 nm (right column) (b).

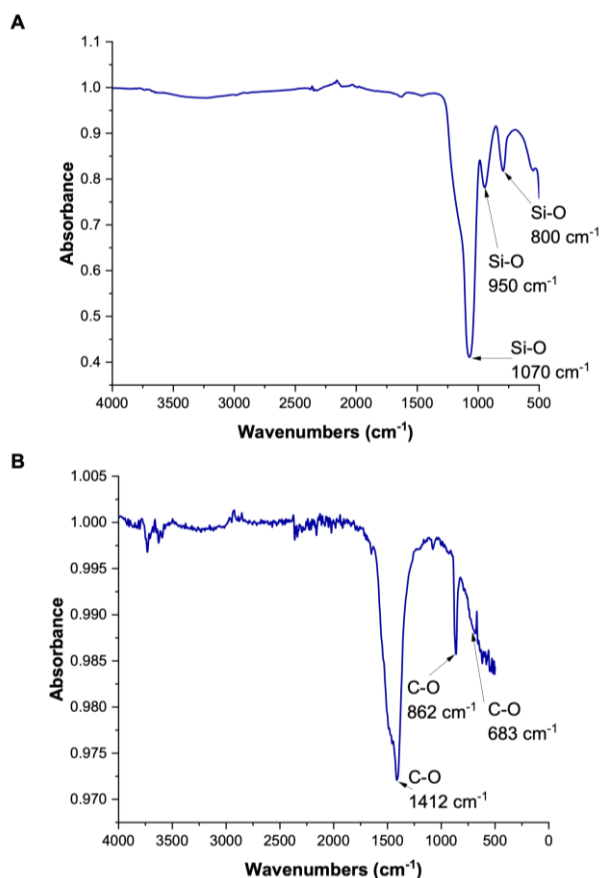


Figure 4 Fourier transform infrared (FTIR) spectra of SiO₂ (a) and CaCO₃ NPs (b).

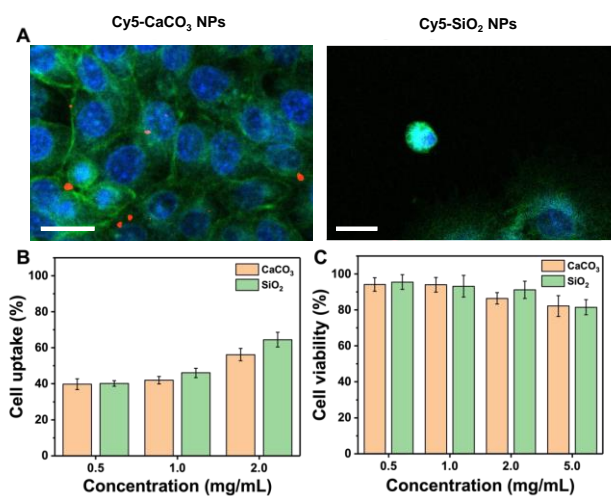


Figure 5 CLSM images of 4T1 cell line uptake incubated with CaCO₃ and SiO₂ NPs at concentration of 2 mg/mL. The cells were costained with AF488 (green) and PI (blue). The NPs were labeled with Cy5 (red). Scale bar = 25 μ m (a). The cellular uptake efficacy of CaCO₃ and SiO₂ NPs (b). Cell toxicity of CaCO₃ and SiO₂ NPs at 0.5, 1.0, 2.0, 5.0 mg/mL concentrations to 4T1 cell line determined by flow cytometry (c).

The percentage of Cy5-labeled CaCO₃ NPs detected in 4T1 tumor tissue was compared with the percentage of Cy5-labeled SiO₂ NPs in the 4T1 tumor sample by using CLSM visualization.

4. Limitations

The main difficulties in developing carriers for drug delivery still include: 1) scaling-up synthesis of NPs for clinical practice, 2) overcoming the accumulation in biological barriers (distribution of NPs in the liver and spleen) [36] and 3) lowering carriers' toxicity. Thus, to reach scalable carrier production, the chosen strategy of using passive drug delivery of NPs without ligand-receptor selection (active targeting) gives more potential advantages for further NPs implementation in therapeutic compositions. Moreover, NPs affinity to various types of tumors is still unknown because of non-comprehensive approach to studying those aspects. However, second challenge in that case became more inevitable to stop the progress. Therefore, investigations in this field would help to find new potential non-toxic, biocompatible NPs to obtain the carrier with adaptive structure and controllable properties.

5. Conclusions

Summing up, we described the synthesis of CaCO₃ and SiO₂ NPs. These NPs were fully characterized. The synthesized NPs exhibited appropriate stability, low toxicity, and biocompatibility. ^{99m}Tc- and Cy5-labeling was performed to investigate the biodistribution of CaCO₃ and SiO₂ NPs. We can propose these NPs as potential carriers for drug delivery. In addition, the proposed synthesis methods are quite simple, cost-effective and easily scalable for further translation into clinical practice.

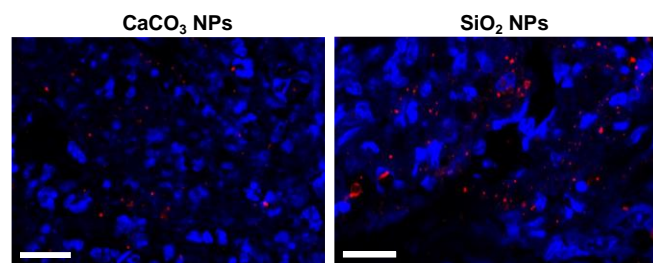


Figure 6 Comparison of Cy5-labeled CaCO₃ and SiO₂ NPs accumulation in 4T1 tumor tissue with fluorescent staining. PI colored cell nuclei and Cy5-labeled NPs visualized as red dots. Scale bars correspond to 200 μ m.

Table 1 Direct radiometry analysis of main organs after injection of CaCO₃ and SiO₂ NPs.

Sample type ^a	CaCO ₃ NPs ^b	SiO ₂ NPs ^c
Heart	3.71±0.6%	3.95±0.3%
Lungs	2.31±0.5%	2.34±0.1%
Liver	82.2±4.2%	83.9±4.3%
Spleen	6.12±0.4%	6.0±0.3%
Kidney	2.31±0.5%	2.96±0.4%
Tumor	1.62%±0.3%	2.8%±0.4%

^a mouse excluded organ type;

^b radioactivity data per gram values (%dose/g);

^c radioactivity data per gram values (%dose/g).

● Supplementary materials

No supplementary materials are available.

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● Conflict of interest

The authors declare no conflict of interest.

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