Review

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Design of Ultrasmall Silica Nanoparticles for Versatile Biomedical Application in Oncology: A Review

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Abstract

Ultrasmall silica nanoparticles, as one type of nanocarriers featured by excellent biocompatibility and efficient renal clearance, are of rapidly growing interest for biomedical applications, particularly in oncology. Undesirably, the intrinsic issues of low site-targeting capability, short circulation time, and limited functionalities of ultrasmall silica nanoparticles severely impede their widespread application in the biomedical domain. Recent researches on surface modification for improved physical properties, enhanced site-specific abilities and multimodality imaging have been continuously emerging, which provide the prerequisite for possible application in the integration of diagnosis and treatment. On this basis, this review summarizes the most widely used synthesis approaches for well-ordered ultrasmall silica nanoparticles with uniform diameter and tunable pore size, and simultaneously highlights the diverse surface functionalization for versatile purposes and biomedical applications, including site-targeted delivery of drugs, stimuli-responsive cargo release, real-time bioimaging as well as cancer theranostics. Finally, the challenges of ultrasmall silica nanoparticles in oncology are further discussed with the aim of promoting their future clinical application.

Keywords: ultrasmall silica nanoparticles; cancer theranostics; multifunctional modification; site-targeted delivery; stimuli-responsive drug release; bioimaging

Introduction

Nanomedicines, in the size ranging from 1 to 100 nm, have been often termed as “magic bullets” for application in cancer therapy [1–3]. Compared with traditional drugs for cancer treatment, nanomedicines exhibit favorable biocompatibility, improved pharmacokinetic (PK) behaviors, and increased tumor targeting ability, thus possessing great potential in drug delivery. Among the organic and inorganic nanocarriers, silica nanoparticles (SiNPs), defined as “Generally Recognized as Safe” (GRAS) by the FDA for more than 50 years, have continued to be considered an attractive and promising nanoplatform in the past few decades due to their low toxicity, high surface area, and stability [3–5]. Besides, the

synthesis of SiNPs is facile, cost-effective, and easy-to-large-scale compared with other nanomaterials. With unparalleled advantages like uniform particle size, tailorable pore diameter, and favorable biocompatibility, SiNPs provide an opportunity for the development of an innovative nanocarrier of various cargoes for the diagnosis and treatment of diseases. However, the long-term sequestration of large-sized SiNPs by the reticuloendothelial system (RES) and the potential accumulation toxicities have given rise to serious barriers to their clinical translation [6].

Efforts have been made to overcome the foregoing challenges associated with the structure complexity of large-sized SiNPs. The study of ultrasmall nanoparticles, with diameters lower than 10 nm, is an area of rapidly growing academic and technological interest as a result of size-dependent properties [7]. Benefiting from the ultrasmall particle size, ultrasmall silica nanoparticles (USNPs) can be easily excreted from the body via renal filtration, which may reduce unwanted accumulation in organs or tissues, thus tackling potential toxicity and stability issues [8]. Moreover, USNPs, as a type of drug delivery nanosystem, can be synthesized by various feasible approaches, in which the physical properties (i.e., morphology, size, and pore volumes) of USNPs can be well controlled and regulated [9].

In addition to intrinsic physical performance, multiple modifications with diverse functionalities on the surface of USNPs can compensate for their insufficiencies like low tumor accumulation efficacy, and further broaden their application in cancer diagnosis and therapy. With efficient *in vivo* targeting ligand decorating strategies and well-developed surface engineering techniques, functionalized USNPs are expected to achieve *in vivo* active tumor targeting, multimodality tumor imaging, biodistribution tracking, and enhanced drug delivery in one single nanoplatform [10].

Despite the rapid development of USNPs, there is still lack of a systematic discussion of USNPs concentrating on the synthesis and biomedical application in oncology. In this review, we provide a concise summary of emerging USNPs towards the controlled design and surface functionalization. On this basis, we further discuss the future challenges and opportunities of USNPs in the integration of tumor imaging and therapy with the intention of accelerating their clinical translation.

Synthesis of USNPs

With the thorough investigation of silica nanoparticles, the methods for their synthesis have been consistently refined. Presently, the principal techniques for creating nanoscale silica are the sol-gel method, template method, vapor phase chemical deposition method, thermal decomposition method, electrochemical method, plasma method, microwave method, and so on. However, some of these methods are not commonly used for synthesizing USNPs primarily due to their high cost and harsh reaction conditions. For instance, electrochemical, plasma, and microwave methods incur high costs and necessitate specialized equipment. Thermal decomposition and vapor phase chemical deposition methods require high temperature or pressure conditions, which are energy-intensive and potentially hazardous. In contrast, the sol-gel and template methods are widely employed for synthesizing USNPs because of their low cost and straightforward and adjustable synthesis conditions. These two methods are commonly used to produce size-controlled USNPs, in which the morphology and pore structure can be rationally adjusted for desired purposes.

Sol–gel method

The sol–gel method, also known as the Stöber method, typically uses silica precursors tetraethyl orthosilicate (TEOS) or tetramethyl orthosilicate (TMOS) as the silica source and methanol or ethanol as the solvent to produce USNPs [11]. Briefly, under acidic or alkaline conditions, the silica ester undergoes a series of chemical reactions to form a stable silica sol system which then gradually reverts to a gel. After drying and sinter curing, USNPs can be obtained from the gel.

In order to endow USNPs with favorable physical properties, some key factors need to be controlled during the process. Maintaining uniformity in the size distribution of nanoparticles is the prerequisite to develop reproducible and large-scale manufacturing processes. Therefore, it is necessary to precisely control the determining factors to obtain monodispersed and uniform USNPs involved in the synthesis process. First of all, the appropriate choice of basic catalysts is of great importance. Compared with $NH_3 \cdot H_2O$, basic amino acids serve as better catalysts as they can accelerate the hydrolysis of

silica (silane) precursors and the condensation of siloxane (Si–O) bonds, thus promoting the formation of highly ordered USNPs [12, 13]. S. Fouilloux et al. prepared monodisperse USNPs in the range of 5–30 nm in one step using arginine as a catalyst and showed that such a catalyst could improve the reproducibility of the reaction [14]. In addition, tuning the reactant concentrations allows the regulation of particle sizes of USNPs in the range of tens to a few hundred nanometers. Of note, the concentration of the silica precursor strongly affects the particle size, with lower concentrations resulting in smaller-sized USNPs [15]. Tadanaga et al. produced USNPs with 10 nm in diameter through regulation of the molar ratio of TEOS:EtOH:H_2O. They also demonstrated an increase in the particle size of silica nanoparticles up to 60 nm with the increase of the TEOS ratio [16]. Temperature primarily affects silica formation by influencing reaction rates. Increasing the temperature accelerates the nucleation rate of the silica source and decreases the condensation rate [17]. In addition, the temperature also affects the particle size of USNPs by affecting the saturation concentration of ammonia. An increase in temperature caused a decrease in the saturation concentration of ammonia, which in turn resulted in a decrease in the hydrolysis and condensation rates and thus a decrease in the particle size of USNPs [18]. Moreover, the particle size of USNPs can be reduced and their dispersion can be improved by adding electrolytes such as sodium iodide. The study conducted by Kim et al. examined the impact of various electrolytes on the size of USNPs. Results indicated that the introduction of sodium iodide caused the most significant reduction in particle size. Even a small quantity of sodium iodide added during synthesis led to a decrease in the particle size of USNPs to 17.5 nm [19]. Therefore, we can conclude that USNPs are typically prepared with TEOS as the silica source and $NH_3 \cdot H_2O$ or amino acids as the catalysts nowadays. To obtain monodispersed and small-sized USNPs, it is critically important to add modifiers, optimize the experimental techniques, and control the reaction conditions throughout the sol-gel preparation process.

The sol–gel technique carries numerous benefits. First, it can yield high purity and uniform structure of silica nanoparticles. Second, it can regulate the morphology of nanoparticles, creating spheres, nanowires, porous bodies, and so on. In addition, the reaction conditions are mild and can be executed at room temperature without utilizing photo initiation or thermal initiation. Moreover, the sol–gel process is suitable for the mass production of USNPs. However, this approach is costly and solely depends on the depletion of precursors to prevent particle growth, resulting in difficulties in achieving the desired particle size range. Additionally, it commonly occurs that the nanoparticles agglomerate and the morphology becomes uneven during the preparation process.

To address the concerns discussed above, multiple adjustments have been proposed to attain controlled size in the synthesis of USNPs. Previous researches presented that USNPs synthesized in the aqueous phase possessed several advantages, including high water solubility and stable fluorescence performance [20]. Using water instead of ethanol as a solvent, Wiesner and colleagues achieved quicker hydrolysis of silane precursors at near-neutral pH levels, as well as better control over the size of USNPs [3]. To further improve the synthesis, a capping agent like polyethylene glycol (PEG)-silane can be introduced as a time interval to halt the condensation reaction and avoid particle aggregation, ultimately leading to smaller and more uniform USNPs [9]. Furthermore, employing heterobifunctional PEG chains facilitates the introduction of accessible ligands onto the USNPs, significantly broadening their applications.

Micelle template method

In recent years, the template method has become a popular technique for synthesizing USNPs with increasing researches on mesoporous silica nanoparticles [21]. The template method is a process that utilizes inexpensive, readily available nanoparticles with controllable shapes as templates. It can yield USNPs with high dispersity and desired shapes/sizes by depositing silica sources on the template surface and then removing the templates [22, 23]. According to the templates used during the synthesis process of USNPs, the technique can generally be classified as a soft or hard template method [24].

As its name suggests, the hard template method uses a substance with a rigid structure as a template, such as carbon nanotubes [25], calcium carbonate ($CaCO_3$) [26], and hydroxyapatite [27]. Both $CaCO_3$ and hydroxyapatite are frequently utilized because they can be eliminated solely through acid treatment, without requiring calcination or organic solvent

treatment. Yuki Nakashima et al. synthesized USNPs with 20 nm in size by employing amorphous calcium carbonate particles as templates [28].

Soft template method typically employs amphiphilic polymers as a template, such as surfactants, microemulsions, and biomolecules. Compared to hard templates, soft templates have rich variety and high variability [29, 30]. The charge interaction with the template facilitates the silica precursor easier deposition onto the template surface. Additionally, soft templates are low-cost, easy to construct, and more suitable for industrialized production [31]. Moreover, soft templates can be formed in various shapes, which signifies that nanoparticles with different structures and properties can be synthesized according to the selected template agent, thus enriching the diversity of USNPs [32].

Currently, the commonly used surfactant templating agents include polyethylene-polypropylene glycol (F127), cetyltrimethylammonium bromide (CTAB), and dodecylamine (DDA). These agents can form micelle templates with uniform particle size when the concentration in water exceeds the critical micelle concentration [33]. At room temperature, the silica precursors hydrolyze in hydrochloric acid, further coagulate and adhere to the surface of the micellar template. With the extension of time, more and more silica precursors grow on the micelle surface, leading to a gradual increase in the particle size of silica nanoparticles. To attain USNPs with an ultrasmall particle size, it is necessary to halt the nanoparticle growth promptly. By employing an organosilane as a capping agent, the silica hydroxyl groups [Si(OH)] located on the surface of USNPs interact with those present on the surface of the organosilane. This reaction leads to a reduction in the number of silica hydroxyl groups present on the surface of the USNPs. The decrease in silica hydroxyl groups benefits the dispersion of USNPs, culminating in the production of uniformly sized and well-dispersed USNPs. In an attempt to decrease the particle size of silica nanoparticles and enhance their biosafety, diverse techniques have been employed to refine the template method for producing USNPs with high dispersibility. Chi et al. investigated the effects of different organosilanes on the morphology of USNPs using F127 as a template. It was observed that the number of methyl groups in the organosilanes as well as their carbon chain lengths affected the particle size of USNPs. Increasing the number of methyl groups and lengthening the carbon chain led to an increase in the spatial resistivity of USNPs, which in turn enhanced the dispersity of USNPs. With trimethylethoxysilane (TMES) as a capping agent, it is possible to reduce the particle size of USNPs to 11 nm [34]. Shimogaki et al. used DDA as a templating agent and investigated the effect of factors (e.g., amount of added DDA, duration of addition, etc.) on the silica nanoparticle size. After adjusting the above factors, they obtained silica nanoparticles with a minimum particle size of 20 nm [35]. Ma et al. synthesized USNPs with improved dispersity and a particle size of 10 nm at pH 8 using the surfactant CTAB as a template, $NH_3 \cdot H_2O$ as a catalyst, and PEG-silane as a capping agent. The CTAB template agent could be removed through dialysis. Furthermore, as shown in Fig. 1, the morphology and particle size of USNPs could be modulated by varying the addition time of the capping agent [36].

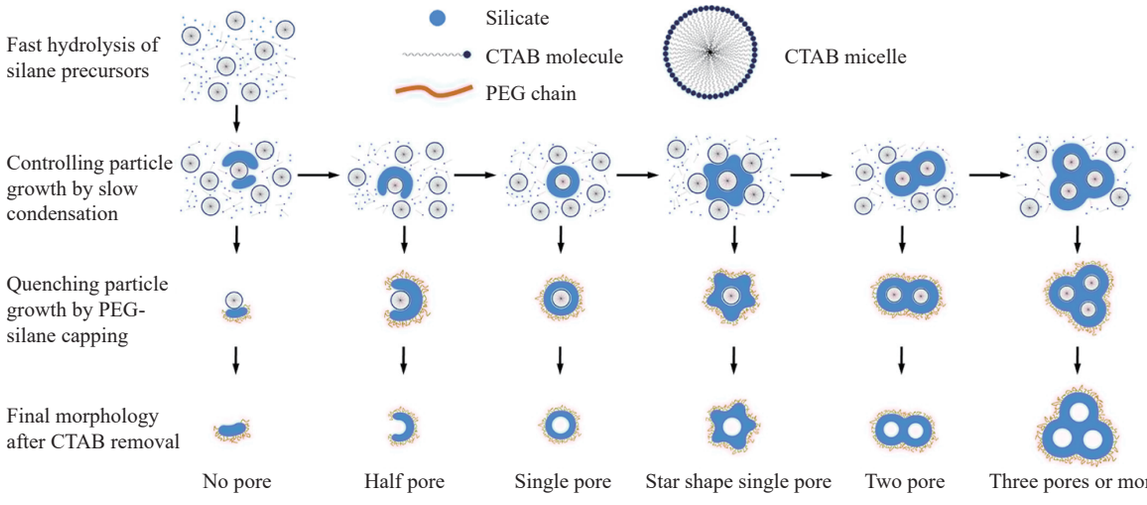

Fig. 1 Diagram of how to synthesize USNPs with various particle sizes and shapes using the soft template method [36]. © 2013 American Chemical Society.

In summary, the template method represents a feasible way to design desired USNPs under a mild reaction condition during a straightforward implementation process. It achieves the controlled synthesis of nanomaterials with diverse morphologies and structures, boasting significant potential for practical applications.

Improved Physical Properties of USNPs by Surface Modification

The behaviors of nanoparticles, both *in vitro* and *in vivo*, are mainly determined by their surface physicochemical properties. The switchable surface charge and accessible functional groups enable the facile synthesis of ultrasmall nanoparticles equipped with small agents like peptides or polymers for distinct properties, including favorable biodistribution and PK profiles [37]. Therefore, it is necessary to analyze, optimize, and control the physical properties and surface chemistry characteristics of USNPs, including morphology, hydrodynamic size, surface charge, surface area, surface functional groups, targeting ligand modalities and densities as well as particle stability [38, 39], so as to further properly control biological activities and safety of USNPs *in vivo*.

Size optimization of USNPs

The ultrasmall size of USNPs can realize rapid clearance and reduce toxicity concerns, but it also hinders sufficient accumulation of USNPs at tumor sites for enough antitumor efficacy. However, with size changing to mimic that of antibodies (hydrodynamic diameter: ca. 12–15 nm), USNPs can effectively resist rapid renal clearance, prolong plasma half-life time, and specifically accumulate at the tumor site via the enhanced permeability and retention (EPR) effect, which combine the advantages of both macromolecular biologics and ultrasmall nanoparticles [40]. PEGylation, one of the most popular modification methods on USNPs, can not only regulate the size by quenching the growth of USNPs but simultaneously endow the nanoparticles with a nearly neutral surface charge, which is also critical to prolong blood circulation and realize desired PK behaviors [36].

Porosity introduction into the USNPs

Porosity introduction into the USNPs benefits the delivery of multiple drugs due to the increased surface area allowing larger amounts of drug encapsulation. With larger surface areas, facile surface engineering can also be obtained for improved physical properties [41] and multiple functionalizations. Nevertheless, other studies suggest that increased specific surface area will simultaneously result in hemolytic activity [42] and cytotoxicity [43]. Therefore, it is necessary to design optimized pore size for reduced side effects and enhanced drug loading capacities.

Biocompatible molecules conjugation onto USNPs

Proper surface modification of USNPs is a prerequisite for potential application and determines the interactions between the USNPs and the environment, which can ultimately affect the stability and circulation time of the nanoparticles. To endow USNPs with desired stability and favorable PK profiles in biological environments, it is indispensable to cover them with biocompatible molecules [10].

Surface modification of silica nanoparticles with hydrophilic polymers like PEG is an essential approach to act as a stealth coating and endow NPs with favorable PK profiles, such as long-term stability, favorable biocompatibility, and systematic biodistribution [44]. During the process, PEG-silane added into the USNP solution facilitated a fast non-covalent association of PEG and silica, which could further promote the condensation of PEG-silane on the USNP surface (Fig. 2) [45]. Notedly, relatively short PEG ligands (molar mass < 1 000 g/mole) were widely used to be decorated on the surface of USNPs because of the insignificant effects of different polymer conformations and thus reduced kinetics complexity of the modified USNPs [46]. Wani et al.

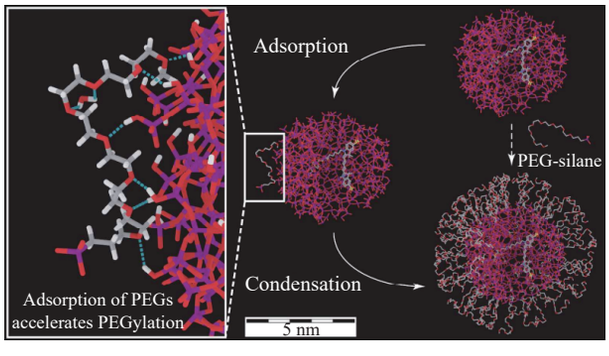

Fig. 2 Schematic illustration of the mechanism of a USNP coated with PEG chains [45]. © 2016 American Chemical Society.

investigated the surface PEGylation of mesoporous silica nanorods, which could prevent dose-dependent hemolysis, improve colloidal stability, and regulate drug loading and release profiles [47].

Besides PEGylation, the biocompatibility and safety of USNPs can also be greatly improved by attaching a lipid bilayer to their surface [10]. The additional layer bound to USNPs by hydrophobic interactions can transfer hydrophobic USNPs to aqueous phase and prevent sensitive USNP core from renal clearance [48]. Additionally, previous researches showed that lipid-coated USNPs exhibited elevated loading capacity, sustained release, and reduced premature leakage of cargoes [49–52], thus regulating the drug release profile in a controlled fashion at the target site. With pro-cellular cationic lipid coating, the modified USNPs can further attain better EPR [53, 54]. For instance, Amin et al. reported a lipid-coated USNP as a drug carrier [49]. With the porous structure on the silica cores, USNPs could offer a large surface area for high content of drugs loading by electrostatic interaction and adsorption. After being coated with lipids, the nanoparticle exhibited better cellular uptake and enhanced bioavailability, as well as reduced premature drug release. Due to better localization to the target site, lipid-coated USNPs showed higher cytotoxic effects on tumor cells and reduced undesired side effects related to premature drug leakage.

Biomedical Application of USNPs in Oncology

Site-specific engineering of USNPs for precise tumor-targeted delivery

Although significant advances have been made in targeting particles design for tumor therapy, technical and regulatory hurdles make tumor targeting continue to remain one of the key challenges of nanomedicines [55]. Compared to the majority of large-sized nanoparticles that depend on the EPR effect for tumor accumulation [56, 57], USNPs, modified with a variety of targeting ligands, exhibit active tumor targeting, specific cellular binding, enhanced receptor binding affinity [58] and receptor-mediated intracellular delivery via internalization, thus achieving the navigation of biological barriers and targeted delivery of loading cargos [59, 60]. Therefore, it is critically important to understand how surface chemical variations affect biological events, PK profiles, and nanoparticle bio interactions (i.e., binding affinity, potency, and toxicity) and take advantage of appropriate multifunctional molecules on the surface of USNPs for enhanced targeted delivery [61].

USNPs, coated with antibodies or peptides to target a receptor specific to cancer, can improve diagnostic accuracy and avoid side effects of off-target reactions. The most widely used approach is to covalently bind cancer-targeting moieties with the end of reproducible PEG chains coated on the surface of the USNPs [62]. Zhang et al. reported 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid (DOTA) functionalized αMSH-PEG-USNPs for the melanocortin-1 receptor (MC1-R) over-expressed melanoma-targeted therapy [63]. With the MC1-R targeting cyclic DOTA-αMSH peptide engineering on the surface, PEGylated USNPs exhibited favorable PK behaviors and targeted tumor uptake and retention. In addition, Chen et al. presented fluorescent core-shell USNPs with $\alpha_v\beta_3$ integrin-targeting peptide embedded and PEG-functionalized, which also showed high target-to-background ratios for $\alpha_v\beta_3$ integrin-expressing human melanoma xenograft models [64].

Although traditional active groups like amino groups ($–NH_2$) or carboxyl groups (–COOH) could be introduced by PEGylation and conjugated with desired target ligands, a proteolytic cleavable linker may be a promising new approach to simultaneously realize surface modification and responsive degradation. ELU001 (NCT05001282), whose clinical data present positive initial safety in a phase 1/2 trial, conjugated USNPs with ~20 molecules of the topoisomerase-1 inhibitor via a proteolytic cleavable linker. For targeting solid tumors overexpressing folate receptor alpha (FRα), ~ 13 folic acid molecules were simultaneously linked [65]. In the preclinical trials, this nanoplatform exhibits remarkable targeting and clear properties due to its multi-targeting ligands decoration and rapid clearance by the renal system [65].

Therefore, we summarize that site-specific engineering on the surface of USNPs will enhance their accumulation at the tumor site and reduce the potential off-target toxicities via various linking methods.

Stimuli-responsive USNPs for controlled drug release

The structure of silica nanoparticles has been proven to be vulnerable by the breakdown of siloxane bonds (Si-O-Si) into biocompatible and excretable orthosilicic acid [$Si(OH)_4$] [66]. However, controlled drug release behaviors and potential accumulation toxicity of large-sized silica nanoparticles at specific sites is still an essential and challenging issue for clinical translation [67]. Compared with large-sized silica nanoparticles, USNPs with small size can realize rapid clearance by the renal system. However, how to realize stimuli-responsive drug release by USNPs is also crucial to further explore pertinent studies.

Grafted with stimuli-responsive (e.g., pH-responsive, redox-responsive, enzyme-responsive, etc.) molecular gates, drug-loaded ultrasmall mesoporous silica nanoparticles are promising candidates for selective release of the cargos in the tumor cells or tumor microenvironment (Fig. 3) [68, 69]. After reaching the weakly acidic tumor microenvironment that is rich in GSH and ROS, the designed chemical bonds within the gate layer disintegrate and expose the cargoes to the external biological environment, thus accomplishing the desired site release of loading drugs. Li et al. introduced OS–N=C–cystamine (Cys)/dialdehyde (DAD) layer on the mesoporous silica nanoparticles to realize responsive cleavage of –N=C– bond and thus realize drug release from the pores in the weakly acidic tumor microenvironment [69]. In addition to pH response, Li et al. conjugated an enzyme-activatable cell-penetrating peptide (CPP) sequence onto mesoporous silica-coated quantum dots (QDs) surface [70]. With an activable CPP containing a nuclear-targeted peptide, the USNPs could sensitively respond to specific tumor protease cathepsin B, one of cleavable enzymes that is frequently encountered in tumors, thus providing a great possibility for controlled release of the loaded antitumor drugs in the cell nucleus.

Another trendy approach for the preparation of degradable organosilica nanoparticles is to introduce biodegradable disulfide (S–S) groups [71, 72], oxamide cleavable groups [73], or enzyme-triggered cleaved amide bonds [74], with the purpose of single or multiple-responsive intracellular degradation and further controlled release of drugs. In this structure, the silane containing the above-mentioned groups was linked to form the cyclic or linear polymer-based organo-bridged alkoxysilanes, which can disintegrate into harmless, small and easily excretable bioproducts under different desired conditions [75], thus realizing selective drug release. For example, as a representative pH-responsive group, S–S bonds within the skeleton break up into sulfhydryl group (–SH) and the structure disintegrates into fragments under the acidic tumor microenvironment, thus realizing specific site release of cargoes.

Molecular probes modified USNPs for enhanced multimodality imaging

Among the most intriguing applications, *in vivo* molecular imaging by NPs, as a non-invasive method to realize diagnosis in one single platform, is the most attractive one [76]. In contrast to cytotoxic semiconductor QDs [77, 78] and easily photo-bleaching organic dyes [79, 80], USNPs have been a more promising nanocarrier of molecular probes for medical applications and particularly *in vivo* bioimaging [81, 82].

USNPs for biodistribution and PK imaging

Biocompatible USNPs, which possess enormous potential to be rapidly cleared *in vivo*, can be regarded as an ideal nanocarrier of biodistribution and PK imaging agents through facile surface functionalization [8]. For instance, USNPs, single or dual-labeled with fluorescent dyes or radioactive elements, allow the collection of reliable information

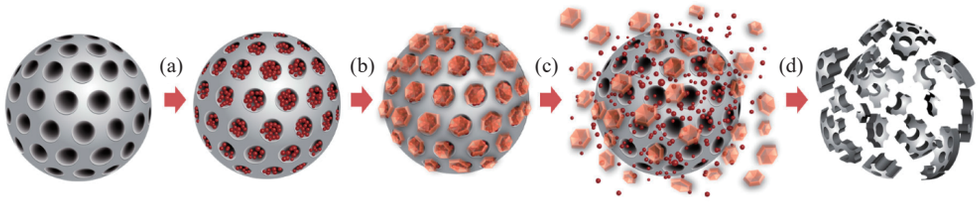

Fig. 3 Scheme illustration of (a) USNPs loaded with multiple drug molecules or fluorescent dyes, subsequently (b) functionalization with gatekeepers to control (c) the release of loaded cargos in mesopores and (d) stimuli-responsive degradation of the nanoparticles [68]. © 2020 Poscher and Salinas.

about their *in vivo* behaviors by optical or/and PET imaging [83].

Chen et al. conducted a screening biological assay on USNPs with varied ligands by labelling with Cy5 [38]. With the fluorescent dye conjugation, the candidates could be monitored and analyzed by real-time fluorescence intensities in terms of their binding affinity, stability, and PK profiles, which was beneficial to select optimum USNPs for clinical trial development. In addition to single fluorescent dye attachment, Licciardello et al. presented USNPs functionalization with a near-infrared dye (Kodak-XS-670) or a radionuclide (^{64}Cu) to realize a detailed biodistribution study *in vitro* and *in vivo* by optical or PET dual-modality imaging [83].

USNPs for target-responsive single and multimodality imaging

Cancer-targeting imaging tools as biomarkers for specially and quantitatively evaluating target status may facilitate molecular classification of tumors and monitor therapeutic effects for effective clinical decision-making and treatment management. However, there is a dearth of tumor-targeting and fluorescence-based multimodality detection tools for enhanced accuracy in the molecular screening and characterization of multiple cancer targets [84]. PET radionuclides, such as ^{68}Ga [85], ^{64}Cu [86, 87], and ^{89}Zr [88], have been used to label immunoconjugates as investigational molecules to image target status and/or monitor treatment response. Nevertheless, unfavorable extended circulation half-lives and physicochemical properties (i.e., dose-limiting toxicities) limit the application of the labelled immunoconjugates in biomedicine. Therefore, controllable biodistribution, sufficient site-targeting ability and simultaneously high detection sensitivity and specificity are the key factors for efficient target-responsive *in vivo* imaging.

Chen et al. reported a ^{89}Zr-labeled anti-HER2-targeting USNP immunoconjugate that was renally-clearable and demonstrated unique biological characteristics for the diagnosis of breast cancer [59]. The USNPs combined site-specific engineered anti-HER2 scFv fragments, radiometal chelators, and aminated USNPs through a multi-step surface functionalization strategy. After multiple modifications, the targeting USNPs could not only enhance accumulation and retention at the target site for improved treatment efficacy but also realize highly specific HER2-targeted PET imaging with substantially reduced off-target accumulation.

In addition to PET imaging, USNPs labelled with fluorescent dyes are also valuable tools to analyze and visualize tumor cells using various imaging modalities including widefield, confocal, and light sheet microscopy. With synthetic dyes loaded inside the silica core of PEGylated core-shell silica nanoparticles, photon yield and fluorophore stability could be substantially improved due to the protection of the nanocarriers for the fluorescent dyes [38, 89]. For example, Chiou et al. synthesized a USNP labelled with infrared dyes Cy3(+), Cy5(+) [90], Cy7(+), and CW800 [91] (see Fig. 4), enabling even deeper tissue penetration for 3D optical imaging and potential *in vivo* tracking of tumor cells spatial interaction.

In terms of *in vivo* multimodal bioimaging, the design of USNPs as a nanoprobe is a promising strategy to realize real-time and noninvasive screening for the *in vivo* distribution of nanoparticles themselves as well as tumor development and progression simultaneously.

USNPs as an all-in-one theranostic nanoplatform for integrated diagnosis and treatment

Since the inception of USNPs, they have been extensively applied in biotechnology as drug delivery systems owing to their favorable biodistribution properties. Although single or dual modification can cover parts of their shortages, such as poor site-specific properties, short circulation half-lives, and low drug loading capacities, it is far from enough to realize theranostics in one single nanoplatform. In contrast, with multiple decorations like fluorescent dyes in the cores and targeting ligands on the surface simultaneously, USNPs can efficiently achieve diagnosis during the treatment process.

Phillips et al. designed an USNP labeled with Cy5 dye, coated with PEG and further decorated with a radiolabeled integrin-targeting peptide ^{124}I-cRGDY [7]. With the modification of Cy5 and 124I-cRGDY, the PEGylated USNP could realize improved physical properties, targeting delivery and multiple bioimaging in one theranostic nanoplatform. Besides, the findings in this study likewise showed favorable *in-vivo* stability, nearly no toxicity, and high cancer staging

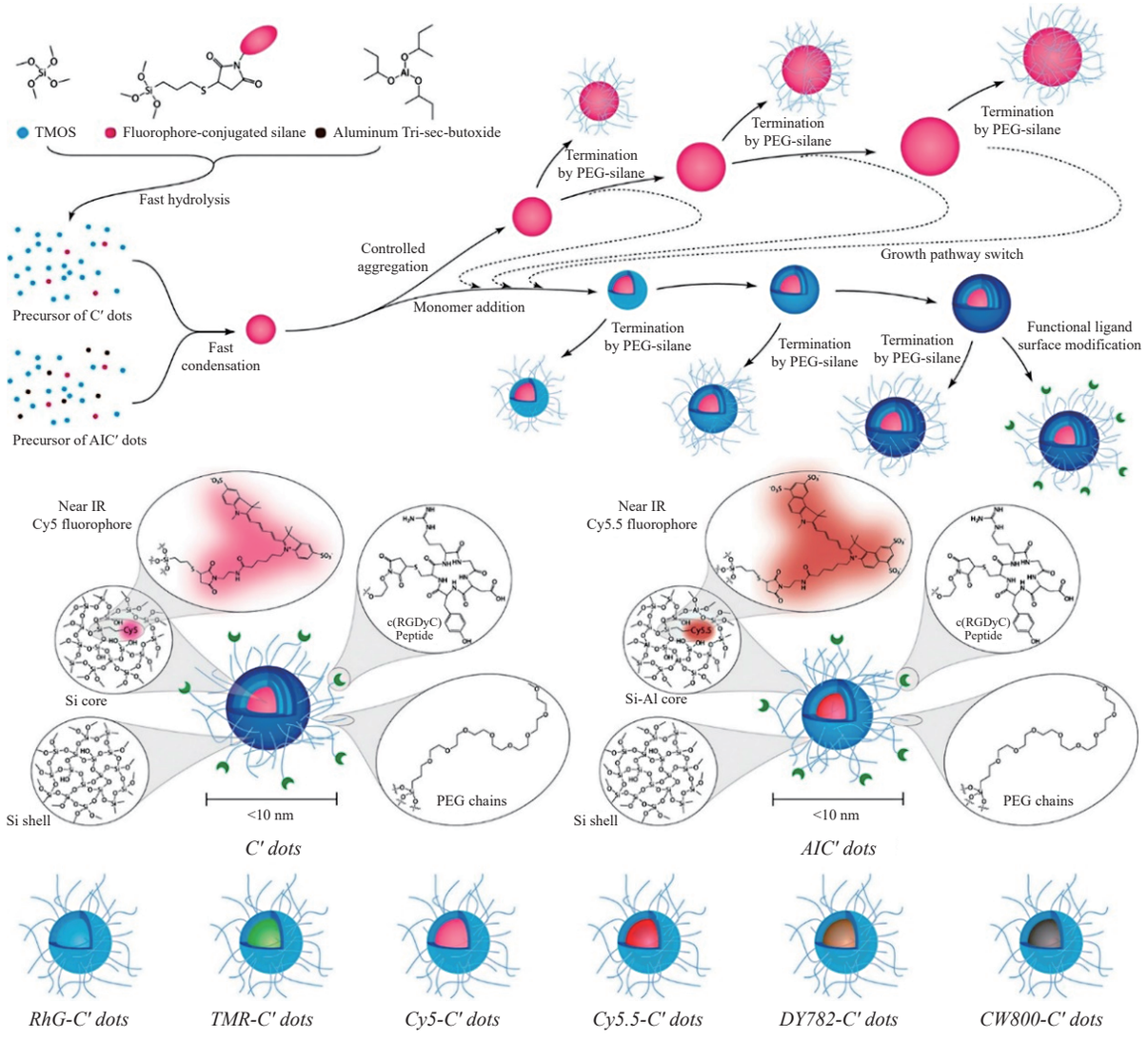

Fig. 4 Scheme illustration of water-based fluorescent USNPs growth pathways, together with the chemical structures of produced particles [9]. © 2015 American Chemical Society.

properties of USNPs. In addition to improving the biocompatible properties of USNPs, PEGylation can also serve as a linker to realize other functionalization, such as the attachment of drug molecules to USNPs [92]. Ma et al. presented multiple types of USNPs covalently inserting silanes with orthogonal groups during PEGylation via a one-pot type synthesis approach [93]. During this process, multiple types of functional ligands were introduced onto the surface of fluorescent PEGylated USNPs by sandwiching heterobifunctional PEG-silanes between the silica cores and functional moieties using different conjugation chemistries (see Fig. 5). Notedly, in this study, a single USNP combined a total of five types of ligand moieties/functionalities, including NIR fluorescent dyes in the USNPs core, peptide ligands, pH-sensitive dyes, radio-metal chelators, and ligand-drug conjugates, thus endowing the particle with theranostic and real-time monitoring capabilities.

Silanization of USNPs is another feasible approach to introduce functional groups. With the abundance of silanol groups on the surface of silica cores, orthogonally reactive ligands like amino- and/or thiol-silane molecules can directly attach to the silica surface through silane condensation, which allows USNPs modification with functionalities like fluorescent dyes and site-specific ligands for particle tracing and tumor targeting. Amino groups on the particle surface are commonly provided by (3-aminopropyl) triethoxysilane (APTES) or (3-aminopropyl) trimethoxysilane (APTMS), which will be free for functionalization. After reacting with the ligand molecules on the silane coupling agents, such as –COOH, hydroxyl (–OH), and $–NH_2$, active targeting modalities (e.g., proteins [94–97], peptides [70, 98, 99], aptamers [100–102], folic acid [103] and

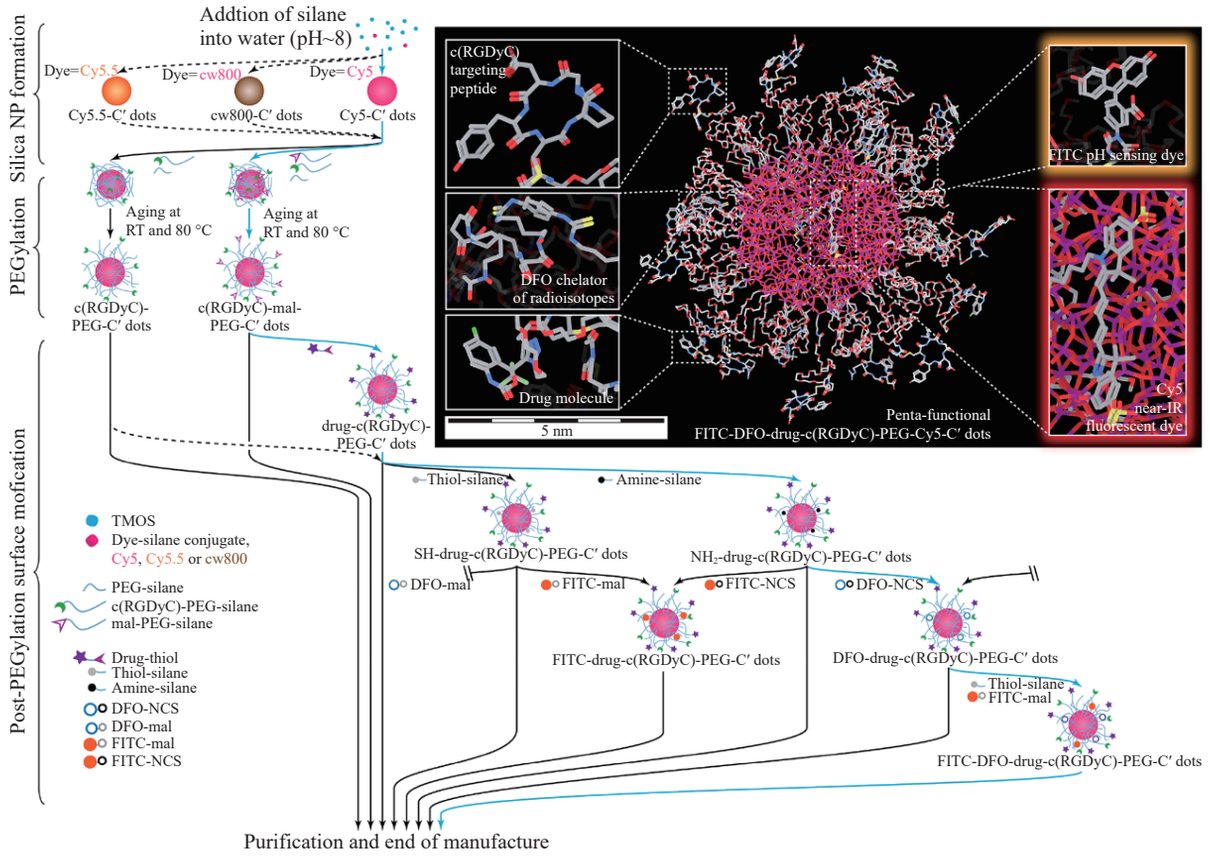

Fig. 5 Scheme illustration of synthesis process of USNPs introduced with PEG for multiple surface modification by an insertion method [93]. © 2017 American Chemical Society.

hyaluronic acid [104], etc.) and imaging molecules (radiolabels [100] or fluorescent dyes [104]) can realize the attachment to the USNPs.

Therefore, it can be concluded that USNPs have the ability to combine diverse functional groups in a single nanoplatform, thus realizing integrated diagnosis and treatment, as well as eliminating unnecessary different classes of agents or surgical interventions.

Conclusions and Perspectives

As one of the most promising nanomedicine candidates, USNPs have attracted significant research attention worldwide in the past few years. USNPs with small size confer distinct biological advantages including rapid renal clearance and low cytotoxicity. Besides, as a flexible drug delivery system, USNPs can realize co-loading and co-delivery of both small molecules and macromolecular drugs in the mesopores or silica matrix for enhanced antitumor therapy efficacy. Beyond the favorable intrinsic physical properties of USNPs, additional functionalities can be introduced by integrating other components, such as organic fluorescent dyes, radiolabeled polymers, biocompatible molecules and site-targeting ligands for advanced cancer diagnosis and treatment.

Although considerable developments have been made over the past years, the integration of multi-functionalities into a single NP platform remains to be challenging but highly desirable. Therefore, it is critically important for a better understanding of reproducible manufacturing processes and changed physicochemical properties *in vivo* after modification. Moreover, standard spectroscopic tools are also needed for long-term particle stability monitoring and abundant functional variations discrimination to select lead candidates exhibiting optimum binding affinity, specificity, favorable PK and stability.

To adequately address each of these barriers, multiple innovative design features can be rationally incorporated into USNPs to generate a new series of nanotheranostics on the concept of quality by design [105], realizing a paradigm shift from nanoparticle-based drug delivery designs to ultrasmall nanoprobes. For example, as for site-specific therapy, it is worthy of extensive efforts to select patient-specific tumor-

associated ligands instead of simple model ligands with insufficient selectivity/targeting capacities [106].

From the perspective of production, although USNPs are relatively safe compared with other nanoplatforms, there still exist some obstacles ahead of translation into the clinic. The validity of bio-safety evaluation is the first issue to be resolved. The difference objectively exists between small-animal models and humans, which results in inadequate evidence to sufficiently prove the reliability of the assessment mechanism. Therefore, it is necessary to mimic the complicated *in vivo* environment and even conduct clinical trials to completely clarify the *in vivo* biodistribution and metabolic behaviors of USNPs. Aside from bio-safety evaluation, the universal production guidelines in good manufacturing practices (GMP) or general standards released by the FDA are merely applicable to conventional nanoformulations. These regulations may not ensure the safety and efficacy of USNPs in the clinic. With heading into the production scale, unpredictable changes within USNPs, including original morphology design, intrinsic physical properties, multifunctionalities as well as biological interactions, may emerge and hinder the pace of the process [107]. Thus, it is critically necessary to draft a feasible and product-focused guideline that individually applies to ultrasmall nanoparticles like USNPs, which will support the progress and intensify the advance of ultrasmall nanomedicines.

In summary, further studies still need to be made to improve nanomaterials properties and resolve concerns of USNPs, thus broadening the application fields and even realizing the translocation of USNP-based nanoplatforms from laboratory to working products for clinical use. In addition, it is also critically important to elucidate the process design criteria for surface modification of USNPs with functional ligands through further research under various reaction conditions, thereby expediting the progress of USNPs ranging from nanoplatform and biosensing to nanomedicine.

CRediT Author Statement

Cheng Zhang: writing-original draft, writing-review and editing, investigation. **Liyuan Zhang**: writing-original draft, writing-review and editing, investigation. **Yuanyuan Ma**: supervision, resources, funding acquisition, writing-review and editing. **Shenghong Ju**: supervision, resources, funding acquisition, writing-review and editing. **Wenpei Fan**: supervision, resources, funding acquisition, writing-review and editing.

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Conflict of Interest

The authors declare that no competing interest exists.

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