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Phototherapeutic spectrum expansion through synergistic effect of mesoporous silica trio-nanohybrids against antibiotic-resistant gram-negative bacterium

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ABSTRACT

The extensive impact of antibiotic resistance has led to the exploration of new anti-bacterial modalities. We designed copper impregnated mesoporous silica nanoparticles (Cu-MSN) with immobilizing silver nanoparticles (SNPs) to apply photodynamic inactivation (PDI) of antibiotic-resistant *E. coli*. SNPs were decorated over the Cu-MSN surfaces by coordination of silver ions on diamine-functionalized Cu-MSN and further reduced to silver nanoparticles with formalin. We demonstrate that silver is capable of sensitizing the gram-negative bacteria *E. coli* to a gram-positive specific phototherapeutic agent *in vitro*; thereby expanding curcumin's phototherapeutic spectrum. The mesoporous structure of Cu-MSN remains intact after the exterior decoration with silver nanoparticles and subsequent curcumin loading through an enhanced effect from copper metal-curcumin affinity interaction. The synthesis, as well as successful assembly of the functional nanomaterials, was confirmed by various physical characterization techniques. Curcumin is capable of producing high amounts of reactive oxygen species (ROS) under light irradiation, which can further improve the silver ion release kinetics for antibacterial activity. In addition, the positive charged modified surfaces of Cu-MSN facilitate antimicrobial response through electrostatic attractions towards negatively charged bacterial cell membranes. The antibacterial action of the synthesized nanocomposites can be activated through a synergistic mechanism of energy transfer of the absorbed light from SNP to curcumin.

1. Introduction

Photodynamic inactivation (PDI) has been emerged as a highly advantageous alternative [1] to antibiotic therapy in the post antibiotic era [2,3]. In some PDI approach, the generation of highly toxic reactive oxygen species (ROS) through photo-excitation of photosensitizer (PS) molecules can not only squelch the bacterial cell components but also detoxify the endotoxins such as lipopolysaccharides (LPS) [4]. Many studies have reported that gram-positive bacteria are more susceptible to certain PS agents through PDI than the gram-negative bacteria [1]. While most of the typical PS molecules could effectively bind and destroy the gram-positive bacterial cells, gram-negative bacteria are relatively resistant to PDI as their complex cell wall limits the permeability of PS [5–10]. Curcumin (Cur) is a polyphenolic natural compound, which has shown phototherapeutic effects [11–13] against gram-positive bacteria [14] and yeasts [15]. However, curcumin shows less PDI effect against gram-negative bacteria, due to cell's outer complex LPS layer and hydrophobic nature of the drug [16]. To overcome this blocking, various strategies have been developed with chemical pre-treatment of bacterial cells using toluene [17] and polycations [18,19], which disorganize the cell wall components. But, its complexity and toxicity limit their in vivo application. In this work, we take a nanocarrier approach with a combination of silver and curcumin-loaded in Cu-containing mesoporous silica nanoparticle (Cu-MSN) as the delivery vehicle to overcome the poor bioavailability of free curcumin.

Silver's antimicrobial activity is well-known since antiquity [20]. In recent times, silver nanoparticles (SNPs) have gained increasing attention in the fields of antibacterial for its release of silver ions. SNPs are extremely capable of interacting with surface bio-receptors, which result in cell internalization and concomitantly generate enormous toxic ROS and interrupt various cellular signaling mechanisms [21,22].

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The latest research has shown that combining silver with antibiotics and other antimicrobials not only reduces the toxicity but also expands their antibacterial effects [23–29]. The mechanisms for the synergistic effects are many, including enhanced surface silver ion release [30], SNP disaggregation [31], improved affinity of the therapeutic drug to bacteria [32], and a combination of intracellular mechanisms to enhance the antimicrobial activity [13,33]. However, the low releasing rate of silver ions and poor aqueous solubility of curcumin remain major limitations for their application in biomedicine [34–37]. To overcome the low bioavailability of larger SNP, researchers have developed hybrid nanoparticles by dispersing silver nanoparticle on various inorganic carriers such as montmorillonite [38], activated carbon [39], zeolites [40] and titanium dioxide [41,42]. Amongst all, porous silica based materials have the advantages such as high surface areas, biocompatibility and allowing control of particle dimensions, morphology and surface functionalization [43–49]. This versatility is valuable to develop effective antibacterial agents via surface modification or encapsulation to deliver the drug molecules at the targeted site. Other researchers have reported the synthesis of SNP-MSN nanohybrids for treating bacterial infections or various cancers [50–58].

In a PDI approach, we will employ the SNP/curcumin combination as dispersed on MSN for two functions; (a) direct sensitization in singlet oxygen generation [59] (b) release of silver ions for anti-bacterial activities. Before, Yoon and colleagues have demonstrated that photosensitization of silver using UV-A and visible light irradiation resulted in significant antibacterial activity enhancement [60]. However, up to the present, very few studies have been explored the phototherapeutic prospective of SNP in medicine [30,61–67]. A plasmonic resonance coupling between curcumin and silver nanoparticle will be exploited to enhance energy transfer of SNP to the photosensitizers [68,69]. Secondly, silver's antibacterial activity is directly proportional to the silver ions released from the delivery system [44,70–75]. Size of SNP plays a crucial role, where cytotoxicity is indirectly proportional to SNP size [76]. So, it is essential to engineering the size of SNP when decorated on MSN surfaces, that are selectively toxic to bacteria with minimal cytotoxic effects to human cells.

Generally, PDI is known to be more effective against gram-positive bacteria than gram-negative bacteria [77,78]. In contrary, SNPs have proved to be most effective against gram-negative bacteria [79–81]. In the current investigation, we demonstrate for the first time that curcumin in combination with SNP and Cu-MSN can significantly enhance the sensitivity of gram-negative bacterial cells towards PDI by enhancing the silver ions release from SNP aiming at synergistic effect with the combination of three antimicrobial agents viz., copper ions, SNPs, and curcumin. Compared to sole curcumin and SNP loaded Cu-MSN, the Cur-Cu-MSN-SNP nanohybrid materials showed excellent PDI against *E. coli* due to the synergistic antibacterial effects of silver, copper ions and curcumin.

2. Results and Discussion

2.1. Physical Characterization

MSN trio-hybrid (Cur-Cu-MSN-SNP) nanocomposites were synthesized using multi-step process. Initially, SNPs were decorated over the surface of Cu-MSN through diamine functionalization (Cu-MSN-NH-NH_2). Subsequently, curcumin was immobilized on Cu-MSN via metal Cu(II)-ligand (Curcumin) affinity (Fig. 1A). In the very first step, the external surface of as-synthesized Cu-MSN was functionalized with diamine-silane (N-[3-(Trimethoxysilyl) propyl]ethylenediamine). The surfactant molecules in diamine modified Cu-MSN (Cu-MSN-NH-NH_2) were then extracted by using ammonium nitrate in isopropanol [82]. Further coordinated with silver ions using silver nitrate and reduced with formalin generate silver nanoparticle on Cu-MSN-NH-NH_2. Before the addition of reducing agent (formalin), a UV visible spectrum showed no absorption peak indicating the absence of SNPs. However, an absorption peak at around 410 nm is observed after the reduction reaction, corresponding to the plasmon absorbance of SNPs (Fig. S1) [83]. In the end, the SNP decorated Cu-MSN was subjected to curcumin immobilization. In addition, another batch of as-synthesized Cu-MSN nanoparticles was subjected to surfactant extraction as stated above and eventually loaded with curcumin (Cur-Cu-MSN).

TEM images revealed highly ordered structures of typical mesoporous silica nanoparticles. The TEM microscopic images of Cur-Cu-MSN-SNP sample shows that SNPs are spherical and uniformly distributed over the Cu-MSN surface (Fig. 1B). Recent studies have assessed the crucial role of the size of SNP in affecting cytotoxicities and found that SNPs (size at around 10 nm) are highly toxic to bacterial cells without affecting HeLa cell viability [84]. From the microscopic images, it is evident that the nanoparticles were uniform after SNP and curcumin immobilization without significant aggregation (Fig. 1B). Fig. S2 revealed the various sample images with color changes after successive modifications in Cu-MSN. The successful impregnation of Cu(II) species in the silica framework was evident from the blue color (Fig. S2-a) and after silver coordination, the nanoparticles attained dark greyish silver color (Fig. S2-b). Further, reduction with formalin resulted in slight yellow color (Fig. S2-c) indicating the formation of SNP [85]. It changed to brownish tinge after curcumin loading in the Cu-MSN-SNP (Fig. S2-d) signifying the successful encapsulation of curcumin. In addition, curcumin encapsulation in bare Cu-MSN resulted in a light yellowish color (Fig. S2-e).

Fig. 2 shows the UV–visible spectra of ethanolic solutions of free curcumin, Cur-Cu-MSN, Cu-MSN-SNP and Cur-Cu-MSN-SNP. Curcumin ethanolic solution exhibited an absorption spectrum at 434 nm (λ_{max}) (Fig. 2-a). It is significant that the absorption peak of Cur-Cu-MSN showed a blue shift to 425 nm (Fig. 2-b), correlated to the n-π^* dipole transition (Keto group in curcumin), which is usually veiled by the solid masking effect of neighboring absorption bands [86]. Therefore, it is conjectured that Cu species in MSN framework would readily create a valance orbital for interaction with curcumin molecules via metal-ligand interactions [82]. SNP loaded Cu-MSN exhibited an absorption peak at 410 nm, (Fig. 2-c) which is due to the surface plasmon resonance of SNPs [87]. In comparison to the sharper Soret band in curcumin ethanolic solution at 434 nm, the curcumin encapsulated Cu-MSN-SNP resulted in homogeneous broadening of the Soret band at 428 nm λ_{max} (Fig. 2-d). The possibility of changes of the Soret band might be the curcumin aggregation inside the mesopore, which evidences the red shift and broadened spectral profile. In addition, it also suggests the important steric effects due to the coordination with copper in the framework [67].

The FT-IR spectrum showed the successive chemical modification in all the samples (Fig. S3). Compared to pristine Cu-MSN, the peak intensity at 960 cm^{-1} (Si—O—H stretch) was reduced obviously after diamine immobilization (Fig. S3-b, c, d), due to the reaction between Si—OH and diamine-silane [88]. The peaks located at 2935 cm^{-1} and 2889 cm^{-1} corresponded to the C—H stretching frequencies of diamine-silane. These results confirmed the successful preparation of Cu-MSN-NH-NH_2 [88]. The curcumin encapsulated Cu-MSN and Cu-MSN-SNP (Fig. S3-d, e) clearly exhibited the similar bands of curcumin (Fig. S3-f) between 1250 and 1600 cm^{-1}, which could be attributed to C—OCH_3 vibration and ν(C═C) stretching vibration of the benzene ring. In addition, the Cur-Cu-MSN samples show low intensity bands in the region of 2900–3000 cm^{-1}, when compared to pristine samples these bands are assigned to the aliphatic C—H stretches. These bands proved the successful grafting of curcumin in Cu-MSN and consistent with the previous report [89].

The mesoporous texture of nanohybrids was further demonstrated by nitrogen adsorption-desorption isotherms using Brunauer–Emmett–Teller (BET) (Fig. 3). Textural information of MSN's structural parameters (i.e., final BET surface area, pore size and pore volume) and changes attained after successive modification are given in Table 1. The surface area and other parameters (total pore volume and pore size) of diamine-modified as-synthesized Cu-MSN, (Cu-MSN-NH-NH_2) after surfactant extraction