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Self-Assembled chitosan/phospholipid nanoparticles: from fundamentals to preparation for advanced drug delivery

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ABSTRACT

With the development of nanotechnology, self-assembled chitosan/phospholipid nanoparticles (SACPNs) show great promise in a broad range of applications, including therapy, diagnosis, in suit imaging and on-demand drug delivery. Here, a brief review of the SACPNs is presented, and its critical underlying formation mechanisms are interpreted with an emphasis on the intrinsic physicochemical properties. The state-of-art preparation methods of SACPNs are summarized, with particular descriptions about the classic solvent injection method. Then SACPNs microstructures are characterized, revealing the unique spherical core-shell structure and the drug release mechanisms. Afterwards, a comprehensive and in-depth depiction of their emerging applications, with special attention to drug delivery areas, are categorized and reviewed. Finally, conclusions and outlooks on further advancing the SACPNs toward a more powerful and versatile platform for investigations covering from fundamental understanding to developing multi-functional drug delivery systems are discussed.

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1. Introduction

1.1. Background

Nanosystems with controllable structures and advanced functions, such as liposomes, micelles, nanoemulsions and nanoparticles, show great power in a wide range of applications, including diagnosis, therapy, in suit imaging and on-demand drug delivery (Barratt, 2000; Sonvico et al., 2005; Bahadori & Mohammadi, 2006; Ochekpe, et al., 2009). Among all the different types of nanosystems, nanoparticles, including silica nanoparticles, carbon nanoparticles, solid lipid nanoparticles, magnetic nanoparticles and polymeric nanoparticles (Table 1), have attracted broad scientific interests in recent years due to its distinct characteristics, such as the abilities to offer high stability and encapsulation capacity of drugs, possibility to deliver both hydrophilic and hydrophobic ingredients, and feasibility for providing multiple routes of administration capability (Sanguansri & Augustin, 2006; Kaur et al., 2008; Chuah et al., 2009; Joshy et al., 2018; Mathew et al., 2018). Several nanoparticle-based drug delivery systems have been introduced into clinical practice, in particular for cancer treatment (Torchilin, 2005; Sato et al., 2006). However, the vast majorities of these nanoparticles either needs complicated fabrication process (such as high pressure, temperature and long operation duration) or involve the utilization of harmful chemicals (such as DMSO and tetrahydrofuran) deemed to be nonbiocompatible, and are therefore not suitable for biomedical applications (Vauthier & Bouchemal, 2009; Battaglia & Gallarate, 2012). Therefore, proposing new methods to fabricate nanoparticles in a more succinct and biocompatible manner is highly appreciated.

To overcome the above-mentioned problems, the recently appreciated 'self-assembly approach' is one of the most promising methods to fabricate nanoparticles without involving any organic solvents or cross-linking agents (Yoo et al., 2005; Cho et al., 2012). This method allows nanoparticles to be generated from individual molecules that are able to assemble spontaneously by electrostatic interactions or noncovalent interactions (Moraru et al., 2003; Schatz et al., 2004; Schug & Lindner, 2005). Besides saving energy, this approach is also more favorable than conventional approaches by protecting the encapsulated bioactive ingredients from harmful stresses involved in conventional fabrication process (Ichikawa et al., 2005).

1.2. Object

Various biocompatible and biodegradable natural polymers show the ability to self-assemble (Li et al., 2014). Among all the choices, chitosan and phospholipid are the well-accepted candidates due to their superior biocompatibility and biodegradability (S Duttagupta et al., 2015; Zhou et al., 2017).

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Table 1. Summary of nanoparticles as drug delivery systems.

Types	Advantages	Disadvantages	Applicatons
Mesoporous silica nanoparticles	Amorphous structure with high porosity and enormous surface area.	Potential toxicity *in vitro* and *in vivo* and certain biohazards.	Deliver anticancer drugs (carboplatin, doxorubicin), Di Pasqua et al., 2009; He et al., 2011 antibiotics (amoxicillin), Li et al., 2010 and heart disease drugs (captopril) Popovici et al., 2011.
Carbon nanoparticles	Enormous surface area; excellent electronic and thermal conductivity; drug degradation protection; release only in specific conditions.	Carcinogenic potential; low biocompatibility and biodegradability; need complex chemical modifications to improve biocompatibility.	Deliver anti-inflammatory drugs (dexamethasone) Luo et al., 2011. anticancer drugs (cisplatin, doxorubicin) Di Crescenzo et al., 2011; Tripisciano et al., 2010 and antibacterial drugs (sulfamethoxazole) Zhang et al., 2011
Solid lipid nanoparticles	Good physical stability; drug degradation protection; controlled drug release; good tolerability.	Low loading capacity; drug expulsion after crystallization; a relatively high water content of the dispersions.	Delivery of lipophobic drugs for dermal (Abdel-Mottaleb et al., 2011), peroral (Muchow et al., 2008), parenteral (Nayak et al., 2010), ocular (Attama et al., 2007), plumonary (Liu et al., 2008), and rectal (Sznitowska et al., 2001) delivery.
Magnetic nanoparticles	Easy handling under external magnetic fields; visualization possibility; enhanced uptake by the target tissue resulting in effective treatment at the therapeutically optimal doses.	Aggregation into larger clusters loses the properties associated with their small dimensions and making physical handling difficult; inadequate magnet system.	Deliver antimetabolites (gemcitabine, 5-fluorouracil) Tong et al., 2011; Arias et al., 2010, anticancer drugs (cisplatin, paclitaxel) Hua et al., 2010; Yang et al., 2006, anti-infective agents (ciprofloxacin) Bajpai & Gupta, 2011.
Polymeric nanoparticles	Incorporation of biodegradable polymers; improved aqueous stability and photostability of the encapsulated drug.	Require chemical modifications with nonionic surfactants to reduce immunological interactions and intermolecular interactions between the surface chemical groups.	Delivery antitubercular drugs (rifampicin) Saraogi et al., 2010, antineoplastic drug (carboplatin) Rejinold et al., 2011, antifungal drug (clotrimazole) Pandey et al., 2005.

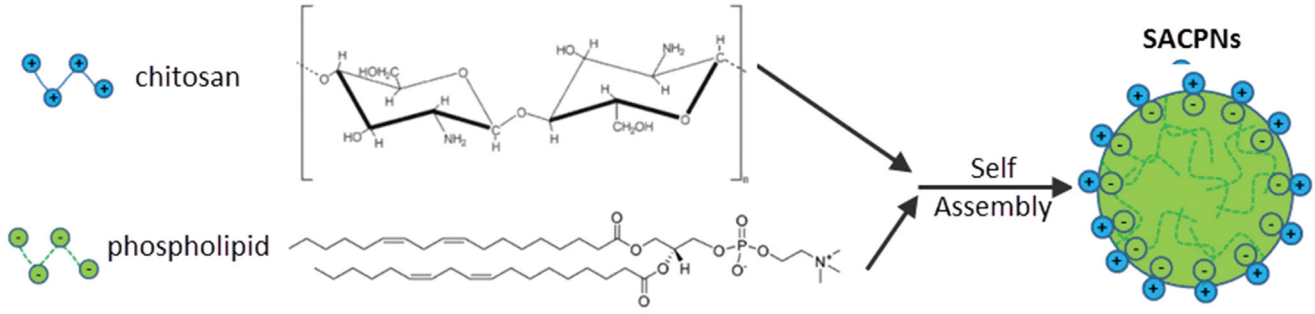

Figure 1. Schematic representation of chitosan-phospholipid interaction and the self-assembly of SACPNs.

Specifically, chitosan is one positively charged natural alkaline polysaccharides derived from chitin that shows nontoxic in both animals and humans with many unique biological characteristics, such as the remarkable bioadhesiveness to mucosal surfaces and the ability to open tight junctions in epithelial cells to strongly promote drug penetration and absorption (Van der Lubben et al., 2001; Panos et al., 2008; Abdolhi et al., 2017; Raza & Anwar, 2017; Eid et al., 2018; Ejeromedoghene et al., 2018; Manuja et al., 2018; Augustine et al., 2019; Dutta et al., 2019). Phospholipids are negatively charged lipid mixtures and act as the basic component of biofilms with various surface activities. They have been widely used in the preparation of various lipid-based drug delivery systems. For instance, Dr. Joshy K. S. has demonstrated the formation of various phospholipid-based nanoparticles for delivering anti-virus drugs and for tissue engineering (Grant et al., 2005; Joshy & Sharma, 2012; Joshy et al., 2017, 2018). Nanoparticles can be formed through the self-assembly between phospholipids and chitosan via electrostatic interactions. Recently, a great deal of interests has been attracted to this self-assembled chitosan/phospholipid nanoparticles (SACPNs), which is the main object that this review would like to introduce, as illustrated schematically in Figure 1.

1.3. Significance

The preparation of SACPNs is quite simple and can be directly prepared by triggering the self-assembly between chitosan and phospholipid; the preparation condition is mild, avoiding the chemical cross-linking agents required for the conventional preparation techniques and the involved tedious operations such as continuous cleaning and continuously performed precipitation (Rodriguez-Hernandez et al., 2005). Moreover, SACPNs have a lipid core and a hydration shell; drugs can be encapsulated in the lipid core by physical embedding or electrostatic interactions with phospholipid. SACPNs can offer high the encapsulation efficiency and superior protection over the encapsulated active drugs. Due to the inherent advantages of phospholipids and chitosan, SACPNs can significantly prolong the residence time of drugs at the action site, promote the penetration and absorption of drugs, and therefore optimize pharmacokinetics (Sahoo et al., 2008). Ever since the SACPNs were first proposed and

demonstrated in 2006, plenty of researches on SACPNs have been carried out and significant progress has been made (Sonvico et al., 2006). Therefore, a comprehensive and in-depth depiction of the whole scene on SACPNs, from fundamentals to preparations for various advanced applications, is desired.

1.4. Contents

The contents of this review include (Yoo et al., 2011; Zhang et al., 2012): (1) the elucidation of the fundamentals of the self-assembly process; (2) the preparation of SACPNs with designed structures; (3) the elaborately characterization of microstructures for SACPNs; and (4) SACPNs with integrated functions for emerging various applications. We present an overview of SACPNs covering from basic principles to progresses made in preparation for advanced applications in recent years.

2. Preparation of SACPNs

SACPNs can be prepared by multiple techniques, such as spray drying, ion coagulation, emulsion crosslinking and solvent injection (Naskar et al., 2019). Among all the methods, the classic solvent injection method is the most convenient and widely applied one, as illustrated schematically in Figure 2 (Sonvico et al., 2006). In detail, the drug is first dissolved in an ethanol solution containing phospholipids, and then the ethanol phase is directly injected into the chitosan aqueous solution under certain stirring speed, subsequently leads to the successful SACPNs formation without involving any intermediate. The results show that the particle size of the generated SACPNs decreases significantly when decreasing the viscosity of chitosan. Moreover, the mass ratio of phospholipids to chitosan has a significant impact over the particle size, zeta potential and stability of the generated SACPNs (Ghosal et al., 2018). When the mass ratio of phospholipids to chitosan is in the range of 5:1–20:1, SACPNs with a small particle size (below 280 nm) and a narrow size distribution can be generated. The formed SACPNs hold a strong positive potential (+40 mV) and good stability. Besides, when conducting the self-assembly in the pH range of 2.5–5.0, the increase of pH leads to the decrease of the positive potential of the SACPNs surface, while the particle size are increased slightly. When the pH is above 5.0, the positive potential of SACPNs decreases rapidly and the particle size increases drastically, which may due to the decrease in the positive charge density of chitosan at high pH values. Therefore, the mass ratio of phospholipid to chitosan, viscosity of chitosan and pH values have significant effects on the particle size and surface charge of the generated SACPNs by conventional solvent injection methods (Sonvico et al., 2005; 2006; Gerelli et al., 2008).

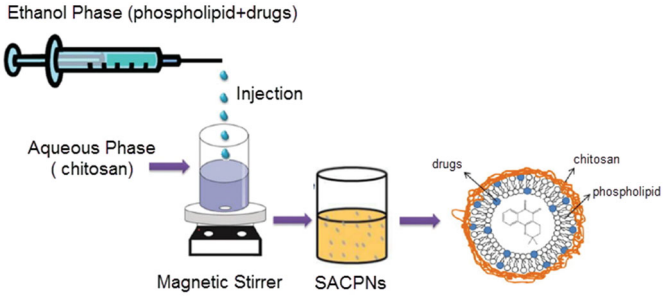

Figure 2. Schematic illustration of the preparation of SACPNs by the classic solvent injection method.

In order to further improve the performance of the generated SACPNs, several modified methods have been proposed and demonstrated. For instance, by adding isopropyl myristate into the ethanol phase, the encapsulation efficiency of the clobetasol propionate can be increased from less than 60% to above 90%. The reason may be attributed to the fact that the addition of isopropyl myristate facilitates the distribution of the clobetasol propionate in the lipid core and thus improve its encapsulation efficiency (Şenyiğit et al., 2010). Besides, tocopheryl propylene glycol succinct is also chosen as the surfactant. The resultant SACPNs show a mean particle size of 95.3 nm, and the entrapment efficiency and drug loading for quercetin are 48.5% and 2.45%, respectively, as shown in Figure 3. Compared with pure quercetin solution, the quercetin-loaded SACPNs showed higher permeation ability, and significantly increased accumulation of quercetin in the skin (Tan et al., 2011). Modified solvent injection method has also been applied to prepare novel insulin-loaded SACPNs. The cryo-SEM results show that the generated SACPNs exhibit a multi-layered cystic structure with an average particle size of 180 nm, an insulin encapsulation efficiency of 94% with a drug loading capacity of 4.5%, which can greatly improve the stability of insulin in artificial gastric and intestinal fluids (Liu et al., 2016).

3. Formation mechanism of SACPNs

The formation mechanism of SACPNs has been investigated in depth and made great progress recently (Park et al., 2004; Quiñones et al., 2018). The Langmuir monolayer technique has been used to characterize the interactions between chitosan and phospholipid at interfaces. Specifically, monolayers of phospholipid were formed on the subphase containing different concentrations of chitosan, and their isotherms were measured. The results showed that chitosan significantly modified the monolayers. Their expansion provided evidence for chitosan binding to the phospholipid. The expansion was found to reach the saturation with increasing chitosan concentration. The degree of saturation of the acid hydrocarbon chain had a significant impact on the architecture developed, namely, the higher was the number of the double bonds, the higher the monolayer expansion at saturation took place. As a conclusion, electrostatic interactions, hydrogen bonding, and hydrophobic interactions were considered as possibly participating in the overall mechanism of chitosan-lipid interactions at interfaces (Wydro et al., 2007). Typically, the electrostatic interactions between negatively charged phospholipids and positively charged chitosan contribute primarily to the formation of SACPNs. For instance, for the prepared SACPNs with an average diameter of

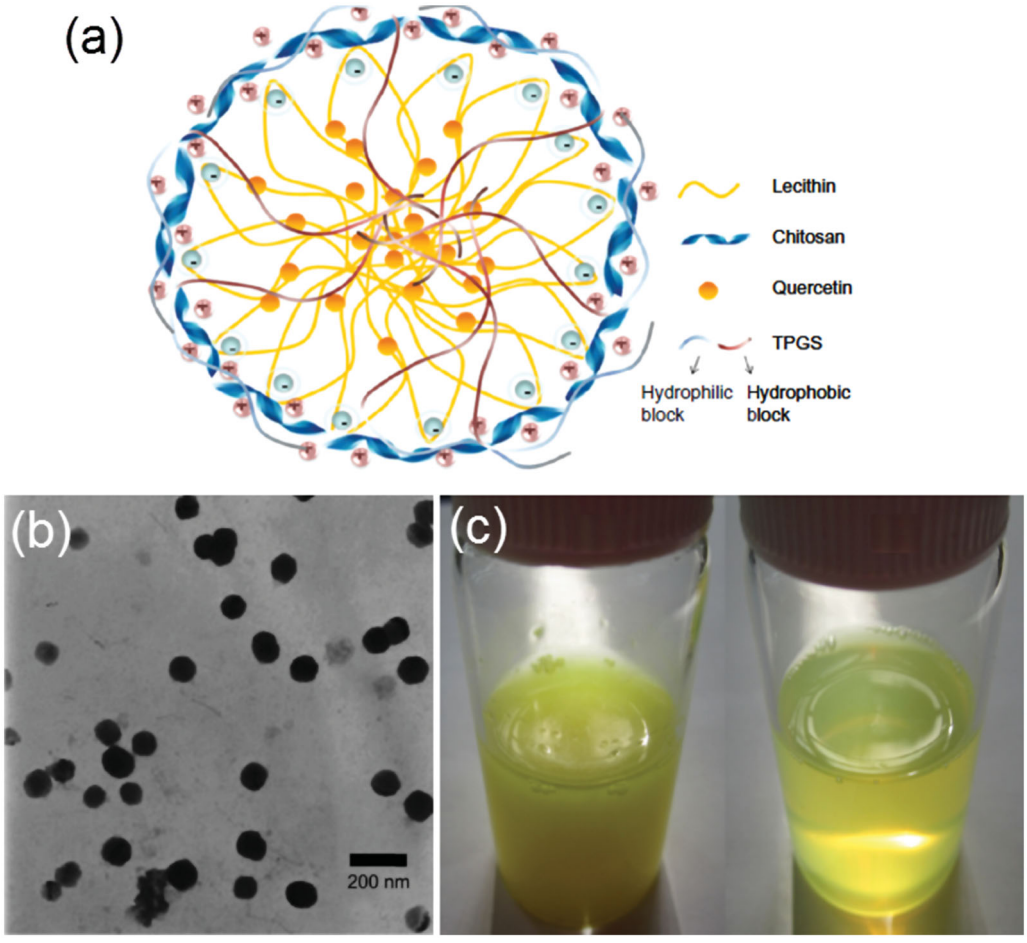

Figure 3. (a) Schematic illustration of quercetin-loaded SACPNs. (b) TEM images of quercetin-loaded SACPNs. (c) Visual observation of crude quercetin in water (left) and quercetin-loaded SACPNs suspension (right). (a, b, c) Reproduced with permission (Tan et al., 2011). Copyright 2011, Informa PLC.

150 nm (Figure 2(a,b)), Fourier transform infrared spectroscopy (FTIR) results show that the shear vibration absorption peak for -NH_2 at 1590 cm^{-1} of chitosan disappears, while the main absorption peak for amide at 1660 cm^{-1} shows no significant change; moreover, the absorption peak for phosphate group in phospholipid shifts from 1236 cm^{-1} to 1217 cm^{-1}, while the absorption peak for the fatty acid chain carbonyl at 1738 cm^{-1} shows no significant change with only a slight decrease in intensity, as shown by the FTIR plots in Figure 2(c), demonstrating that the positively charged functional group in chitosan interacts with the polar portion of the phospholipid to induce the self-assembly formation (Sonvico et al., 2006). Moreover, studies by neutron scattering have also confirmed the strong electrostatic interactions between phospholipid and chitosan. Specifically, SACPNs were prepared, freeze-dried and re-hydrated in a D_2O atmosphere. Then the neutron scattering were performed in the temperature range of 20–50 K using the backscattering spectrometer. The comparison of SACPNs in the dry state with similar ones at an hydration level of about 0.3–0.4 (g D_2O/g hydrated sample), as shown in Figure 4(d) Sonvico et al. 2006. The comparison results indicate that the presence of an outer chitosan 'coating' reduces the mean square fluctuations of the hydrogen bonding in the lipid component, thus leading to a stiffer nanostructure.

Besides, SACPNs encapsulated with drugs are also used as models for investigating the formation mechanism. For instance, for SACPNs loaded with natamycin, the FTIR results show that the shear vibration absorption peak for –NH_2 at 1590 cm^{-1} of chitosan disappears, and the absorption peak of the phosphate group in the phospholipid shifts from 1245 cm^{-1} to 1216 cm^{-1}, while there are no interactions between natamycin and the chitosan or phospholipid, as shown by the plots in Figure 2(e) Bhatta et al. (2012). These results demonstrate that in an acidic medium, the amino groups of the chitosan can be protonated with positive charges and can interact with the phosphate group of the phospholipid through the non-covalent bond-charge interactions to form stable SACPNs spontaneously (Lawrie et al., 2007; Chuah et al., 2009). During the self-assembly process, drugs can be loaded into the SACPNs by means like dissolution, physical embedding and chemical bonding (Goldberg et al., 2007).

4. Characterization of SACPNs microstructures

The microstructure of SACPNs has a significant impact on its functional properties, such as encapsulation efficiency, release behavior *in vitro* and *in vivo*, and storage stability. Transmission electron microscopy (TEM), atomic force microscopy (AFM) and neutron scattering results show that the

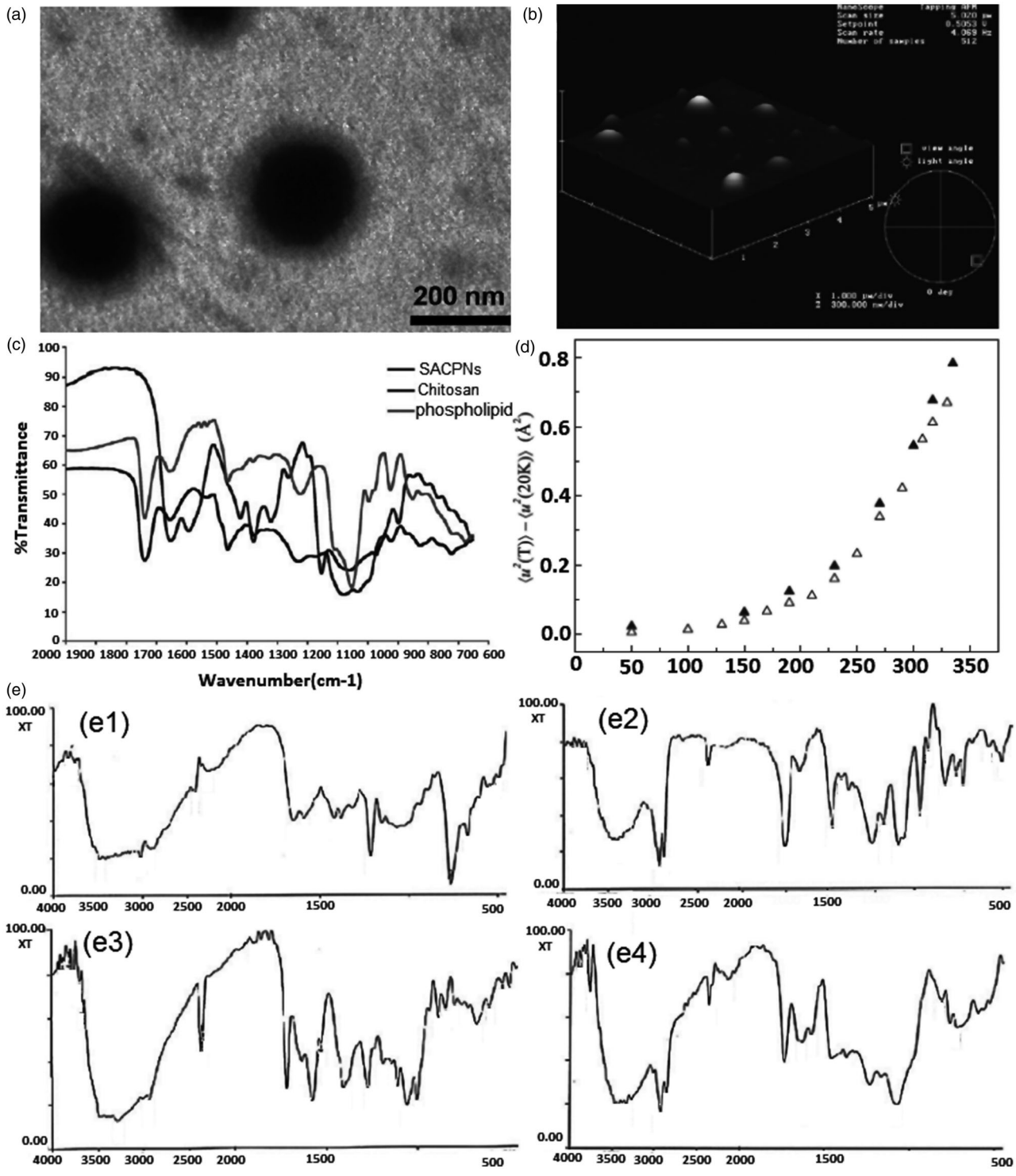

Figure 4. (a) Transmission electron micrographs of SACPNs. (b) Atomic force microscopy images of SACPNs. (c) FTIR spectra of SACPNs and of the two components separately. (d) Comparison of the mean square hydrogen fluctuation differences of a dry SACPNs (open triangles) and a pure phospholipid one (closed triangles). (e) FTIR spectra of (e1) chitosan, (e2) phospholipid, (e3) natamycin and (e4) natamycin-loaded SACPNs. (a, b, c) Reproduced with permission (Sonvico et al., 2006). Copyright 2006, Elsevier. (d) Reproduced with permission (Sonvico et al., 2006). Copyright 2006, Elsevier. (e) Reproduced with permission (Bhatta et al., 2012). Copyright 2012, Elsevier.

SACPNs have a spherical core-shell structure, as demonstrated in Figure 4 and illustrated schematically in Figure 5(a). Particularly, the interior is a dense core mainly composed of phospholipid, providing an ideal place for the encapsulation and delivery of hydrophobic drugs, such as curcumin (Pathak et al., 2015), artesunate (Chadha et al., 2012), diflumethasone valerate (Özcan et al., 2013), and clobetasol propionate (Şenyiğit et al., 2010), and their encapsulation efficiency in SACPNs can be as high as 80%. The extranuclear is a positively charged hydration shell, which is beneficial to exerting the unique biological characteristics of chitosan. Moreover, mesoscopic simulation has been applied

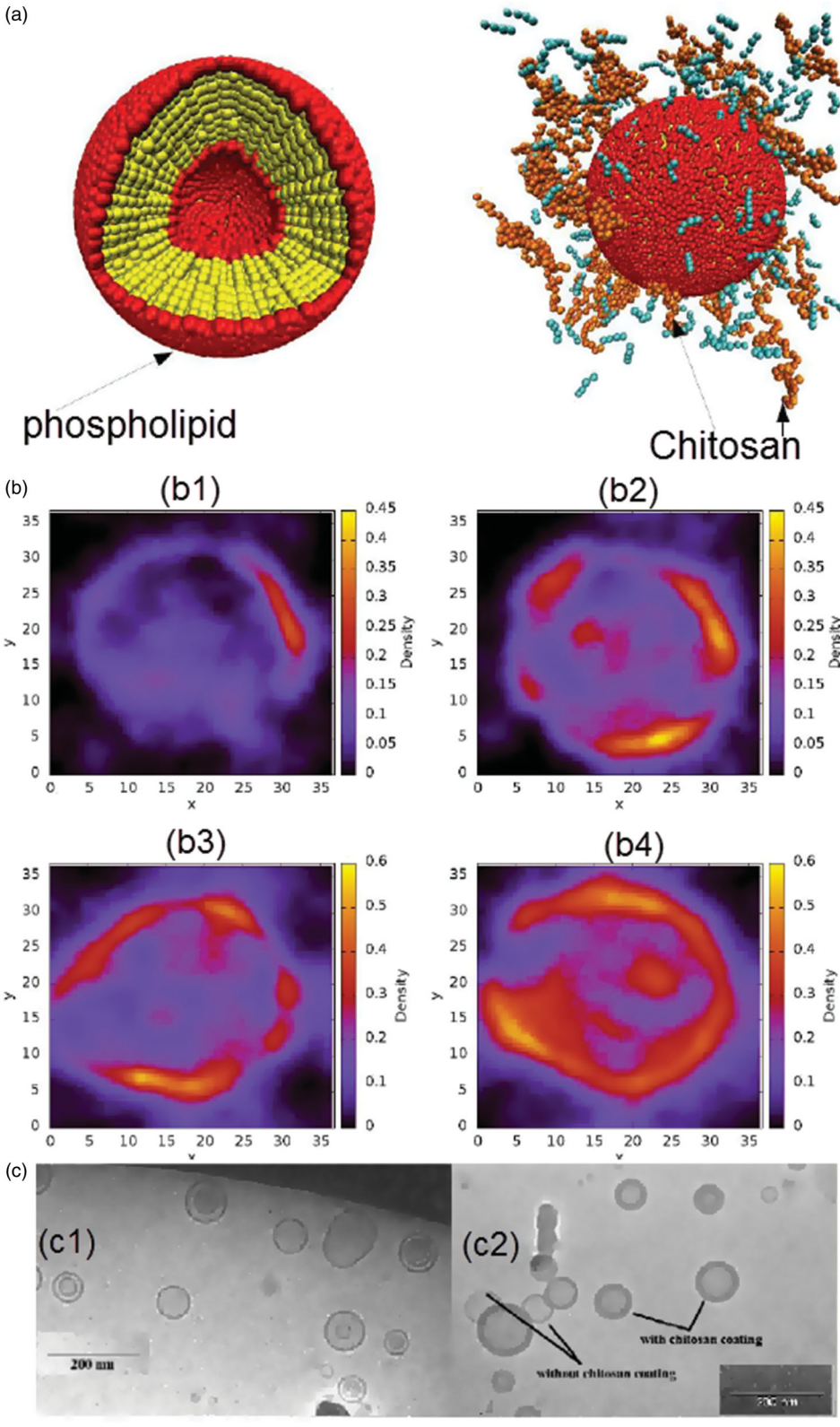

Figure 5. (a) Schematic illustrations of the mesoscopic models of SACPNs. The yellow spheres represent the hydrophobic part of the phospholipid, while the red spheres represent the hydrophilic part. The chitosan are assembled in a random configuration around the phospholipid. (b) Distribution of chitosan on the xy plane. (b1) 50 chains of chitosan; (b2) 100 chains of chitosan; (b3) 150 chains of chitosan; (b4) 200 chains of chitosan. (c) Cryo-TEM image of SACPNs containing tamoxifen (c1) and progesterone (c2). (a, b) Reproduced with permission (Terrón-Mejía et al., 2018). Copyright 2018, MDPI. (c) Reproduced with permission (Gerelli et al., 2008). Copyright 2007, IOP Publishing.

to study the self-assembly behavior and the structure of SACPNs. It is found that phospholipid and chitosan spontaneously form stable nanoparticles, and the drug can be encapsulated in the lipid core, while chitosan distributes around the exteriors of the SACPNs, as shown by the simulated schematics in Figure 5(b) Terrón-Mejía et al. (2018).

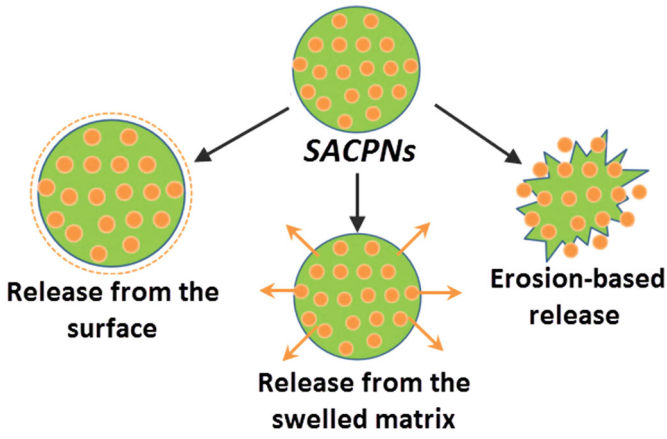

Figure 6. Schematic illustrations of drug release mechanisms of SACPNs.

Moreover, the microstructures of SACPNs are also closely related with the hydrophobicity and charge properties of the encapsulated drugs. For instance, when loading the uncharged hydrophobic progesterone into SACPNs, the formed nanoparticles are with a single-chamber vesicle structure composed of a lipid bilayer and surrounded by an outer layer of chitosan, as shown in Figure 5(c), which is similar to the structure of unloaded blank SACPNs. However, when loading the positively charged hydrophobic tamoxifen, the microstructure of the formed nanoparticles is diversified. Specifically, the microstructure is changed into a single-chamber vesicle with a particle size of 20 nm formed by a chitosan-coated phospholipid layer, as well as a multi-chamber vesicle with particle size around 80 nm, and the vesicles are composed of the lipid bilayer and chitosan which are alternately separated and multi-layered. Most of the tamoxifen (above sixty percent) is loaded into multi-compartment vesicles and is distributed in multiple layers of lipids, with only less than thirty percent into single-chamber vesicles (Gerelli et al., 2008).

5. Drug release mechanism of SACPNs

The drug delivery mechanism of SACPNs is critical for their applications described afterwards and deserved to be stated in detail (Narayanan et al., 2014). There are mainly three types of release mechanisms of SACPNs: release from the surface, erosion-based release and release from the swelled matrix, as illustrated schematically in Figure 6 (Nathanson et al., 2018). The release of drugs from SACPNs is a complicated process and typically comprises not only one mechanism (Jourghanian et al., 2016). For instance, when the drug-loaded SACPNs are subjected to physiological environment, the encapsulated drugs on the surface of SACPNs will be first released, leading to the 'burst release' phenomenon. Gradually, the chitosan shell of SACPNs would be eroded by the physiological environment, resulting in the release of drugs from the shell. Besides, different stimuli from the physiological environment, such as pH changes and ionic strength changes, would weaken the electrostatic interactions between chitosan and phospholipid, leading to the swelling of SACPNs and triggering the release of drugs encapsulated in the lipid cores of SACPNs (Son et al., 2017).

6. Emerging applications for SACPNs

SACPNs create an excellent eco-friendly environment for delivering active components, which can be harnessed in many applications including the drug delivery, production of cosmetics and foods (Chaouat et al., 2017), tissue engineering (Shanmugam & Banerjee, 2011), cell sorting and capture, biochemical assays (Zhang et al., 2008). Here, we limit our discussion to the progress made in recent emerging applications including drug delivery systems, advanced foods and antibacterial field.

6. 1. Advanced drug delivery systems

Ever since the SACPNs were proposed and fabricated, the applications of SACPNs in drug delivery systems have been extensively studied. Various active pharmaceutical ingredients (APIs) have been encapsulated with optimized encapsulation efficiency, drug loading, particle sizes and unique characteristics, as summarized in Table 2 (Hafner et al., 2011; Barbieri et al., 2013; Chhonker et al., 2015; Moreno et al., 2015; Clementino, Batger, et al., 2016; Liu et al., 2016; Clementino, Pozzoli, et al. 2018; Alkholief, 2019; Khan, 2019; Terrón-Mejía et al., 2018). Due to the remarkable biocompatible and biodegradable of SACPNs, it can be used as ideal drug vehicles for transdermal, mucosal, ocular and oral drug delivery. It is worth mentioning that the biocompatibility and bioactivity of SACPNs are extremely important when used for drug delivery. The biocompatibility and bioactivity of SACPNs are typically investigated by MTT assay and hemolysis test *in vitro*. Moreover, intraperitoneally administration into mouse is used to investigate the acute toxicity *in vivo* and biodistribution test is also applied to identify whether it has good biocompatibility and bioactivity (Cardoso et al., 2018; Nosrati et al., 2018; Yuan et al., 2018). Among all these techniques, MTT assay is the primary one that is typically used as the first test to investigate the biocompatibility and bioactivity of SACPNs.

6.1.1 Transdermal delivery

Nanocarriers have appeared as an effective strategy for transdermal drug delivery due to the overcome of steric obstruction and protection of active ingredients at both extracellular and intracellular environments (Liu et al., 2015). SACPNs can promote percutaneous penetration of drugs therefore can be used as desirable platform for mucosal drug delivery. The improvement of percutaneous penetration is closely related to the unique property of chitosan. Specifically, adhesive chitosan helps to prolong the residence time of the drug on the skin surface and facilitate the skin hydration and swelling. At the same time, chitosan has the ability to reversibly open the tight junctions between keratinocytes and weakening the barrier layer of the stratum corneum. Moreover, phospholipids can be well fused with the lipid components

Table 2. Summary of recent studies of SACPNs as drug delivery systems.

APIs	Encapsulation efficiency (%)	Size of SACPNs (nm)	Drug loading (%)	Characteristics	Application	References
Tamoxifen citrate	81.3 ± 2.1	94.8 ± 7.2	30.2 ± 0.3	Release can be triggered by enzymatic attack of lipase and lysozyme.	Oral administration	Barbieri et al., 2013
Insulin	94.69 ± 0.20	128.3 ± 9.45	4.60 ± 0.16	Improve oral bioavailability, time-dependent release, and therapeutic activity of insulin.	Oral administration	Liu et al., 2016
Melatonin	7. 1 ± 0.2	122.8 ± 0.8	4.8 ± 0.2	The SACPNs can be stored in solid form for 7 months while retaining physico-chemical properties and the encapsulated drug.	Oral administration	Hafner et al., 2011
Capsaicin	96.80 ± 0.59	18.04 ± 0.95	96.0	Simulation investigation about the interaction between capsaicin and CS is very weak compared to that with lecithin.	Transmucosal administration	Terrón-Mejía et al., 2018
β-lapachone	52.4 ± 2.4	330.1 ± 3.5	25.8 ± 7.4	Can obtain no parasite reduction while stop the lesion progression.	Transdermal administration	Moreno et al., 2015
Clobetasol propionate	92.2 ± 0.5	248.25 ± 15.1	10.9	Use of SACPNs in chitosan gel can significantly improve the risk-benefit ratio.	Transdermal administration	Şenyiğit et al., 2016
Simvastatin	98.52 ± 1.33	146.7 ± 26.2	90.7 ± 0.87	Enzyme triggered drug release and permeation across mucosal surfaces through physiological biodegradation processes.	Nasal administration	Clementino et al., 2016, 2018
Amphotericin-B	74.6 ± 0.75	282.7 ± 5.2	5.71	Achieved higher AUC and MRT in comparison with marketed formulation.	Ocular administration	Chhonker et al., 2015
Doxorubicin & piperine	45.96 ± 2.63 for DOX; 52.91 ± 3.56 for PIP	157.7 ± 4.53	8.93 ± 0.82 for DOX; 8.46 ± 0.57 for PIP	Co-delivery and sustained release of DOX and PIP, with the potential to be used to treat MDR cancer cells.	Multiple administrations	Alkholief, 2019
Cisplatin	89.2 ± 0.5	181.0 ± 0.43	2.11 ± 0.7	Controlled delivery of cisplatin in cancer therapy.	Multiple administrations	Khan et al., 2019

in the skin, both of which are beneficial for the drug to penetrate the skin from the surface (van der Lubben et al., 2001). For instance, clobetasol propionate-loaded SACPNs with a particle size around 250 nm are generated, as shown in Figure 7(a,b). The anti-inflammatory activity is then evaluated using carrageenan-induced hind paw edema test on rats, and histological analysis is performed to evaluate the possible presence of morphological changes, as shown in Figure 7(c). The results indicate that SACPNs-in-gel formulation show significantly higher edema inhibition compared to other formulations tested. Furthermore, histological analysis of rat abdominal skin shows neither morphological tissue changes nor cell infiltration signs after application of the formulations. Taken together, the results show that the use of SACPNs in chitosan gel as a drug carrier significantly improves the risk-benefit ratio as compared with sodium-deoxycholate gel and commercial cream formulations of clobetasol propionate (Şenyiğit et al., 2016). Besides, SACPNs for dermal delivery of diflucortolone valerate (DFV) that would maintain the localization in skin layers without any penetration have also been prepared. The results of rat skin permeation test show that the SACPNs-based gel can significantly increase the retention time of the drug on the skin, especially the stratum corneum and epidermis, and the retention amount is 6.33 times that of diflumiconic acid valerate cream, without any penetration, as shown in Figure 7(d). *In vivo* pharmacodynamic results show that the anti-inflammatory effect of the SACPNs-based gel is better than that of diflumiconate valerate cream, and there is no change in the skin barrier function, as demonstrated by the plots in Figure 7(e) Özcan et al. (2013).

6.1.2 Mucosal delivery

Due to the mucoadhesive property of chitosan, SACPNs have been used extensively as a carrier for mucosal drug deliver (Baltzley et al., 2014), which can facilitate the drugs to act onset rapidly, avoiding the first pass effect, bypassing the blood-brain barrier and entering the central nervous system. Therefore, SACPNs can perform as vehicles for drugs that are not suitable for oral delivery with low bioavailability. Moreover, the positively charges on the surface of SACPNs are inclined to interact with the negatively charges on the mucus surface, which further facilitates the penetration of drugs through mucosal epithelial cells *in vivo*. For example, SACPNs loaded with simvastatin are prepared for nasal mucosal administration as a novel approach to deliver the statins to the brain. The optimized SACPNs can have an average diameter of 200 nm, positive surface charge (+48 mV) and a particular high encapsulation efficiency of simvastatin around 98%. The encapsulated simvastatin can be released in a quick manner *in vitro* with around 35.6% released in

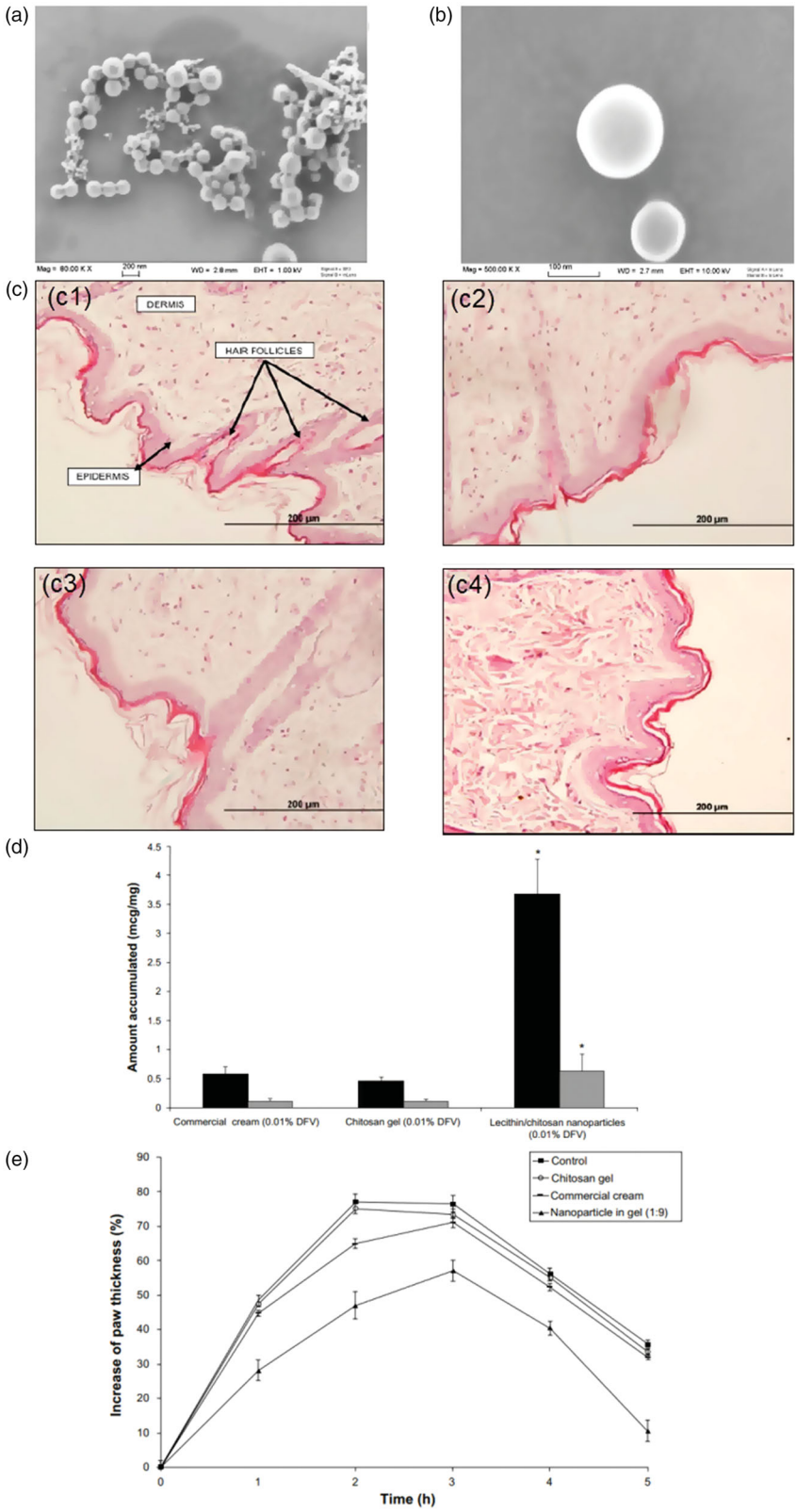

Figure 7. (a, b) SEM images of the clobetasol propionate-loaded SACPNs at 80 Kx (a) and 500 Kx (b) magnifications. (c) Histology of the rat skin samples: (c1) control; (c2) commercial cream; (c3) SACPNs; (c4) Na-DOC gel. (d) Amount of DFV accumulated from formulations in the SC + epidermis (dark bars) and dermis (light bars). (e) Changes in the paw thickness of the rats with respect to time after formulation applications. (a, b, c) Reproduced with permission (Şenyiğit et al., 2016). Copyright 2017, MDPI. (d, e) Reproduced with permission (Özcan et al., 2013). Copyright 2013, Dove Press.

6 hours, as shown in Figure 8(a). Moreover, the MTT test results of human nasal epithelial cells show that the SACPNs have good cytocompatiblity while the cytotoxicity of simvastatin-loaded SACPNs (IC_{50}) is found to be three times lower than the simvastatin suspension. *In vivo* studies have shown that after intranasal administration of simvastatin-loaded SACPNs, the 25% of delivered simvastatin can be distributed in the brain and the kidney, with few distribution in other

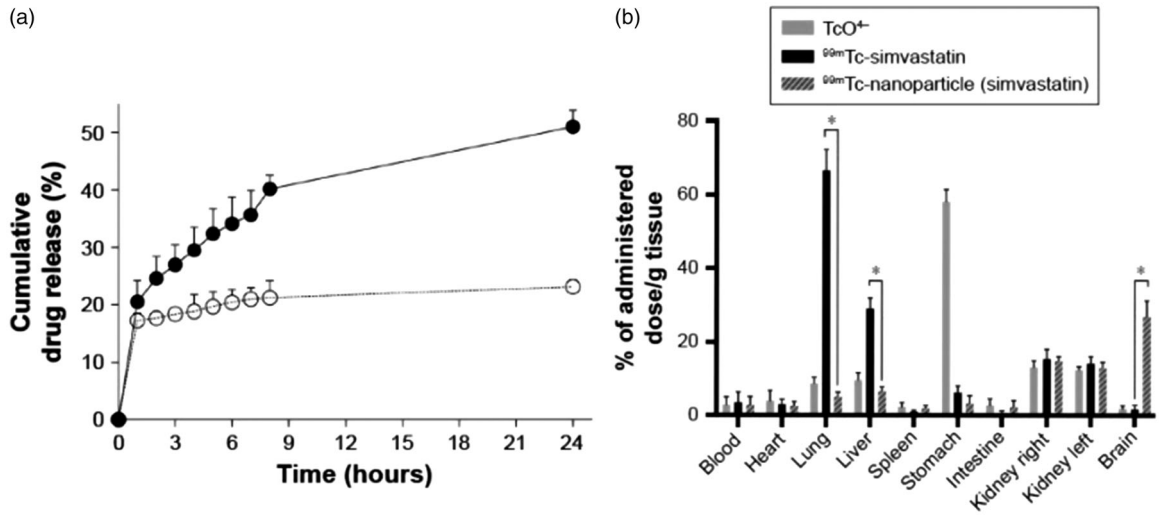

Figure 8. (a) Simvastatin release profile from simvastatin-loaded SACPNs (filled circle) and a control simvastatin suspension (open circle) in simulated nasal fluid (b) Radioactivity biodistribution in rats 90 minutes after the nasal instillation of 20 μL (10 μL in each nostril) of 99 mTc-labeled simvastatin-loaded SACPNs, simvastatin suspension, and pertechnetate (TcO_{4-}) (a, b) Reproduced with permission (Clementino et al., 2016). Copyright 2016, Dove Press.

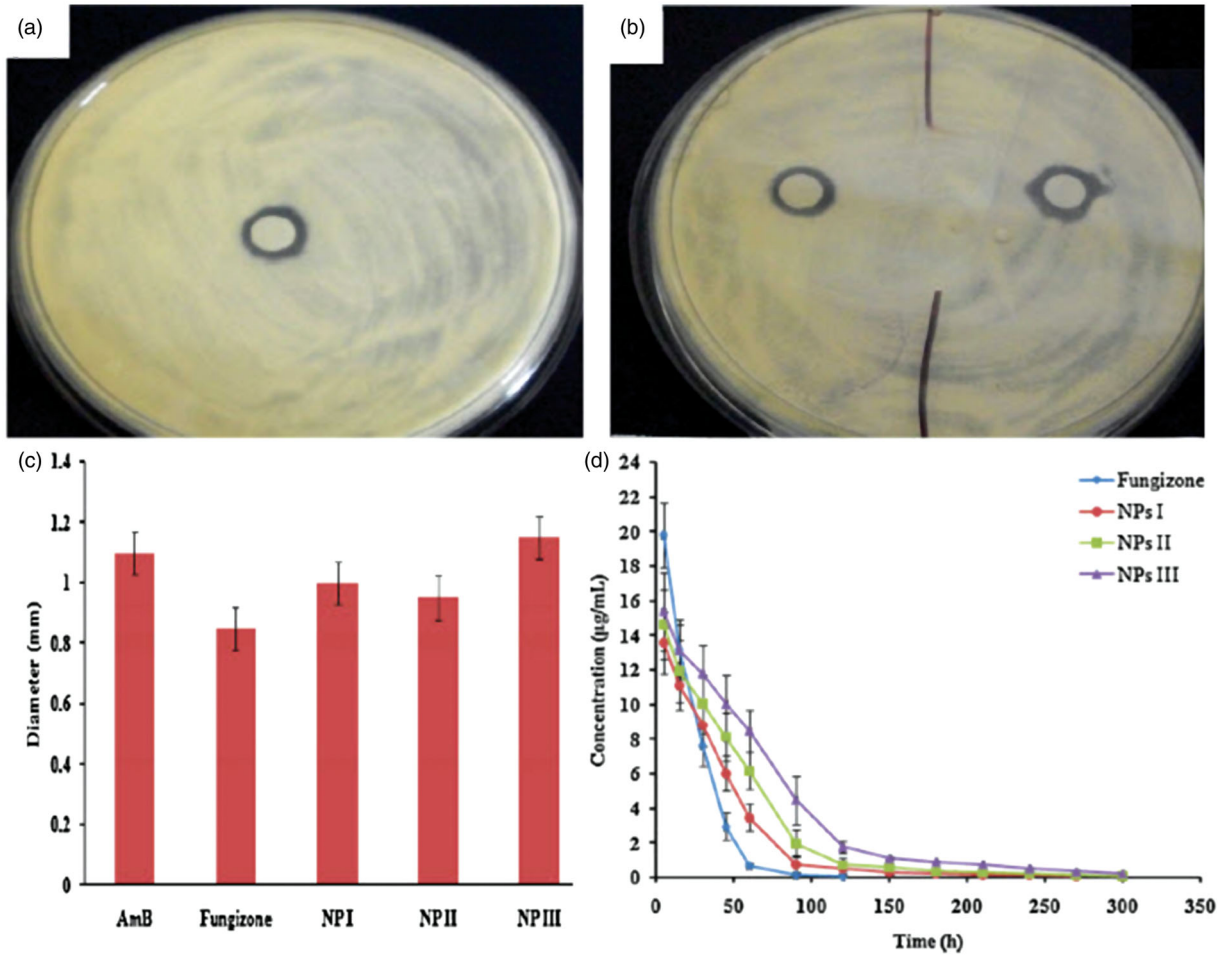

Figure 9. (a, b) Zone of inhibition of amphotericin-B, Fungizone and amphotericin-B-laded SACPNs against A. fumigates. (c) Bar graphs represent zone of inhibition of different formulations against A. fumigates. (d) Cular pharmacokinetic profile of amphotericin-B following topical instillation in rabbit eyes. (a, b, c, d) Reproduced with permission (Chhonker et al., 2015). Copyright 2015, Elsevier.

tissues; while for the simvastatin suspension, the drug is mainly distributed in the lung, liver and kidney, but is rarely distributed in the brain, as demonstrated by the plots in Figure 8(b). The results demonstrate that the SACPNs overcome the blood-brain barrier and significantly increase the distribution of the drug in the brain through the nasal cavity to the brain, with good biocompatibility and cytocompatibility (Clementino et al., 2016).

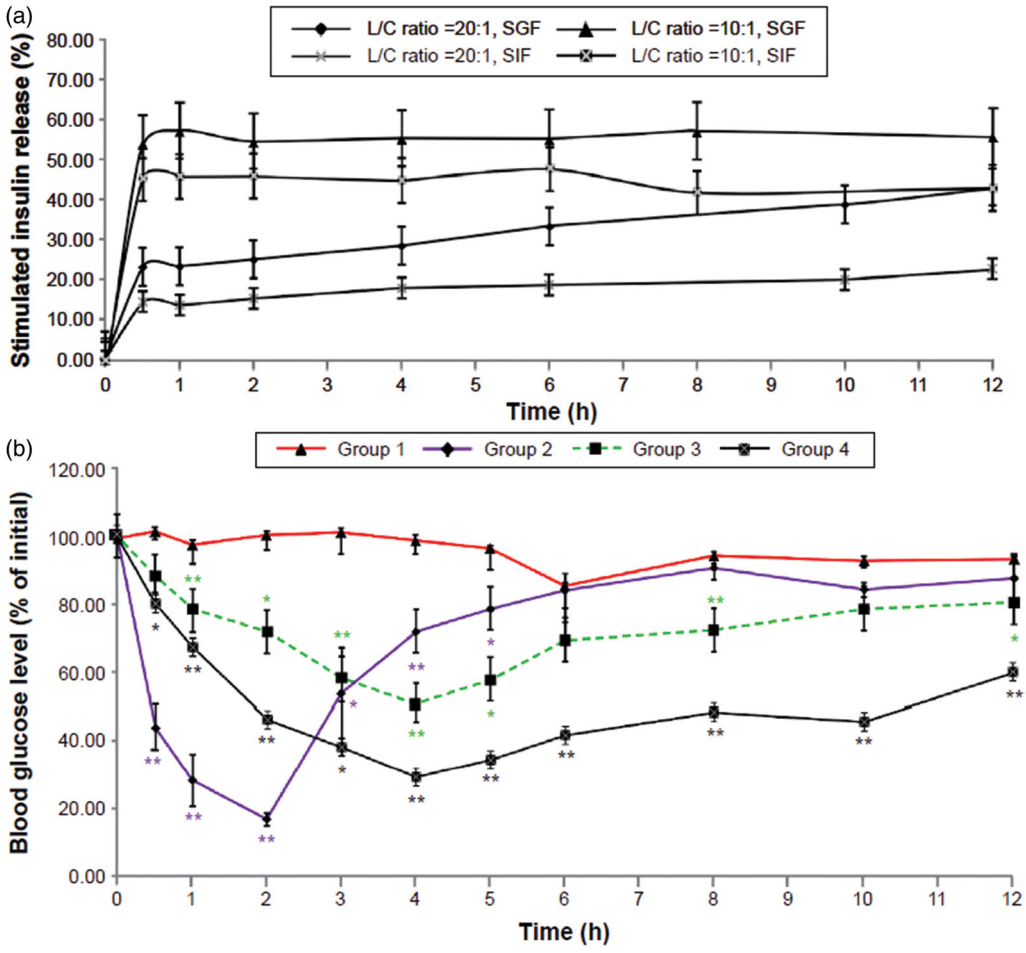

Figure 10. (a) In vitro time-dependent release profiles of insulin-loaded SACPNs with different phospholipid/chitosan ratios. (b) Time-dependent reduction in blood glucose levels in diabetic rats. (a, b) Reproduced with permission (Liu et al., 2016). Copyright 2016, Dove Press.

6.1.3 Ocular delivery

Chitosan can form disulfide bonds with mucus glycoprotein on the mucus gel layer, therefore improve precorneal retention and enhance the interaction with eye mucosa (Partenhauser & Bernkop-Schnürch, 2016). Moreover, chitosan also holds other special characteristics suitable for ocular drug delivery including nontoxic, low eye irritation, mucoadhesive, in situ gelling, transfection and permeation enhancing properties. All of these advantages contribute to the applications of SACPNs as suitable ocular deliver vehicles (Kapanigowda et al., 2015; Werle & Bernkop-Schnürch, 2008. Fox instance, amphotericin-B is encapsulated in SACPNs for prolonged ocular application (Chhonker et al., 2015). The prepared SACPNs are in the size range of 161.9–230.5 nm, entrapment efficiency of 70–75% with positive zeta potential of 26.6–38.3 mV. Moreover, the minimum inhibitory concentration (MIC) and inhibition zone size of the antibacterial *in vitro* are compared with commercial products. As demonstrated by antifungal susceptibility against Candida albicans and Aspergillus fumigatus, amphotericin-B-laded SACPNs exhibit pronounced mucoadhesive properties, as shown in Figure 9(a, b and c). *In vivo* pharmacokinetic studies in rabbit eyes indicate the bioavailability and precorneal residence time can be improved by 2.04 folds and 3.36 folds, respectively, as demonstrated by the plots in Figure 9(d). Therefore, the prepared amphotericin-B-laded SACPNs are of great significance for prolonging the acting duration of amphotericin B in eye medication and improving patient compliance.

6.1.4 Oral delivery

Oral administration is the most convenient, least invasive, and minimum hepatic uptake among all the drug administration methods. However, the bioavailability of orally administered drug is always damaged because of their instabilities in the gastrointestinal tract and inefficient absorption due to low permeability across biological membranes. SACPNs have excellent mucoadhesiveness, which is beneficial to prolong the residence duration of the drug in the gastrointestinal mucosa; at the same time, SACPNs can open the tightly-packed intestinal epithelial cells, therefore can significantly improve the oral bioavailability of the drug. For instance, insulin-loaded SACPNs can be prepared through a modified solvent-injection method (Liu et al., 2016). Generated insulin-loaded SACPNs have a mean size of 180 nm, an insulin-entrapment efficiency of 94%, and an insulin-loading efficiency of 4.5%. *In vitro* analysis revealed that insulin-loaded

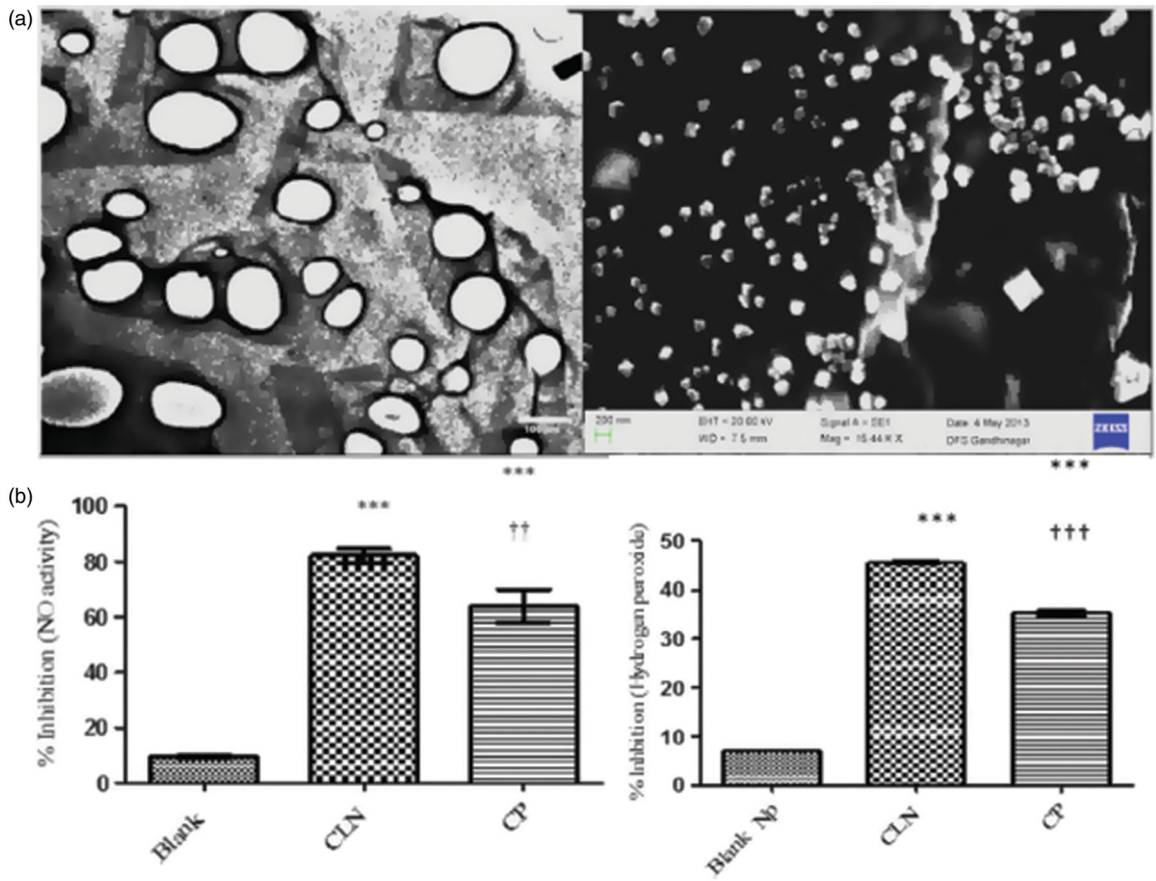

Figure 11. (a) Morphological characterization of curcumin-loaded SACPNs by TEM (left) and SEM (right). (b) The inhibition of Nitric oxide activity (left) and H_2O_2 activity (right) in blank nanoparticles (Blank), curcumin powder (CP) and curcumin-loaded SACPNs. (a, b) Reproduced with permission (Pathak et al., 2015). Copyright 2015, Springer Nature.

SACPNs that are orally administered to streptozotocin-induced diabetic rats can exert a significant hypoglycemic effect, as demonstrated by the plots in Figure 10(b). The relative pharmacological bioavailability following oral administration of SACPNs can be 6.01%. Therefore, by applying SACPNs, some hydrophilic peptides, such as insulin, can be successfully entrapped and delivered orally with improved oral bioavailability, time-dependent release, and therapeutic effects.

6.2 Other promising applications

6.2.1 Functional foods

Besides using as advanced drug delivery vehicles, SACPNs can also be applied as platforms for production of functional foods. For instance, curcumin encapsulated SACPNs can be obtained through solvent-injection method, as illustrated by the microscopic images in Figure 11(a) Pathak et al. 2015. The *in vitro* antioxidant lipid peroxidation (TBARS), radical scavenging (DPPH, NO, H2O2, reducing power) activity assays of powdered curcumin and curcumin-loaded SACPNs are performed. It is found that the generated curcumin-loaded SACPNs are roughly spherical in shape, present high positive zeta potential (>30 mV) and stable in the pH range of 2–6. Moreover, they have enhanced antioxidant ability in comparison to curcumin aqueous suspension, as shown by the plots in Figure 11(b). The curcumin-loaded SACPNs have great potential as functional food ingredient of natural origin.

6.2.2 Antibacterial

Flavonoid compounds are strong antioxidant and antibacterial agents, but their applications are hampered due to their poor dissolution and bioavailability (Gupta et al., 2013; Ruesgas-Ramón et al., 2017). The use of SACPNs for the encapsulation and delivery of antibacterial agents has received increasing attentions. For instance, kaempferol (KAE) is loaded into SACPNs and compared with pure KAE to determine antifungal activity against the phytopathogenic fungus, as illustrated schematically and shown by the microscopic images in Figure 12(a and b) (Ilk et al., 2017). KAE can be successfully encapsulated in SACPNs with an efficiency of 93.8 ± 4.28% and the generated SACPNs show good physicochemical stability. Moreover, *in vitro* evaluation of the KAE-SACPNs is tested by the release kinetics, antioxidant and antifungal activity in a time-dependent manner against free KAE. Encapsulated KAE exhibited a significantly inhibition efficacy against Fusarium oxysporium during 60 days' storage period, as demonstrated in Figure 12(c). The results indicate that KAE-SACPNs can be used as suitable antibacterial agents and could solve the problems related to the solubility and loss of KAE during use and storage.

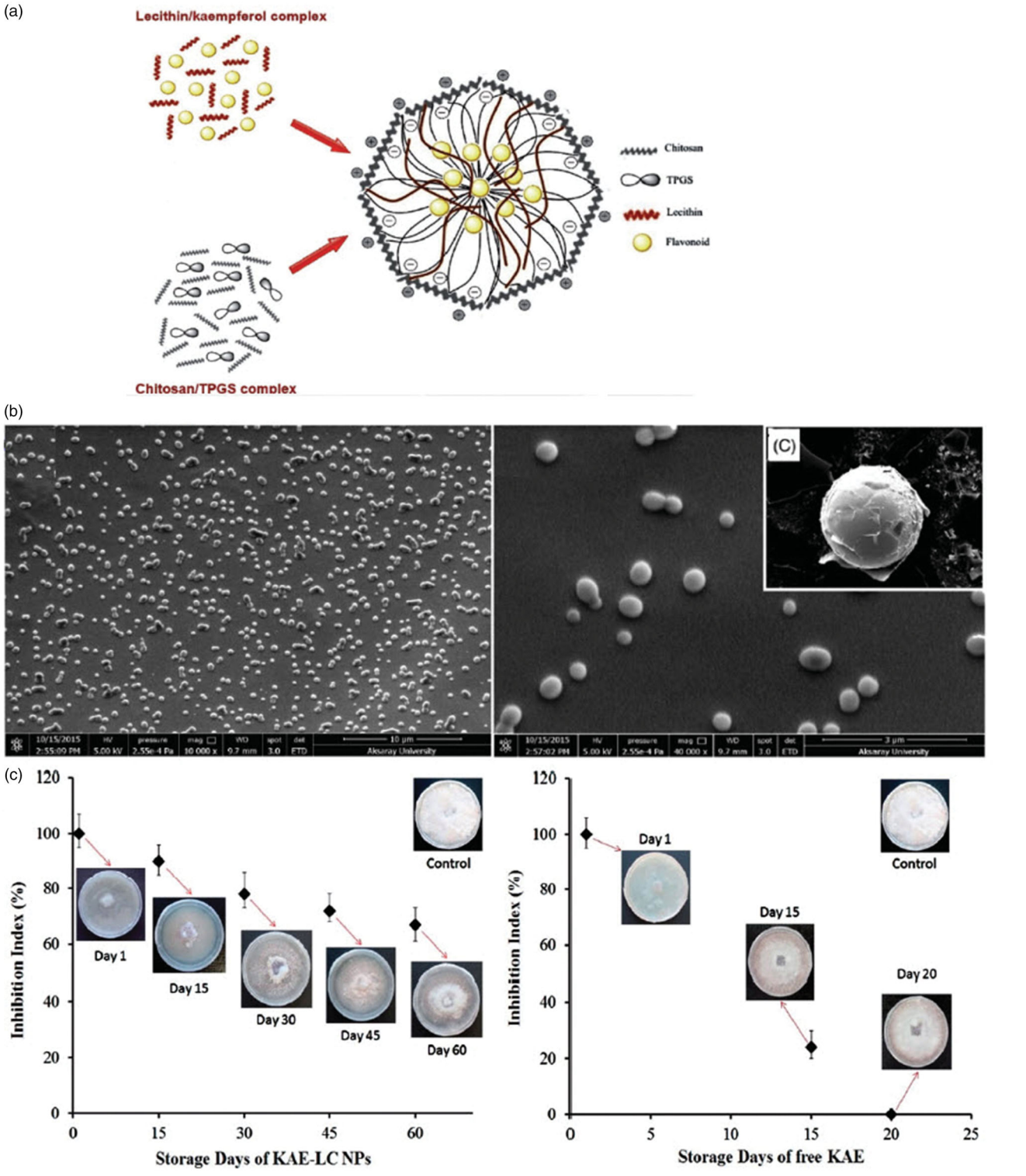

Figure 12. (a) Schematic illustration of KAE-SACPNs. (b) Morphology and dispersivity of KAE-SACPNs. (c) Effect of KAE-SACPNs and pure KAE on the radial growth of Fusarium oxysporium in time dependent manner. (a, b, c) Reproduced with permission (Ilk et al., 2017). Copyright 2016, Taylor & Francis Group.

7. Conclusions and outlooks

7.1. Conclusions

The novel self-assembled chitosan/phospholipid nanoparticles (SACPNs) break through the limitation of drug hydrophobicity on vehicle construction, not only can improve the encapsulation efficiency, but also can increase the stability against the surrounding enzyme attack. The SACPNs are easy for preparation, simple for scale-up production, and can have high encapsulation efficiency by optimizing the generation process. SACPNs can have superior gastrointestinal mucosa and transdermal penetration, which have attracted widespread attention and can be used in a broad range of applications in drug delivery.

7.2. Outlooks

While a great number of SACPNs have been generated and applied in multiple areas, most SACPNs perform their functions based on the natural bioadhesion of chitosan and the regulation of tight junctions on epithelial cells, and therefore leave large spaces for further investigations. There are three major directions for the future developments of SACPNs that we believe worth stating and sharing with scientists who are interested in SACPNs: (1) proposing novel techniques to fabricate SACPNs; (2) investigating modifications to chitosan and phospholipid to functionalized SACPNs; (3) personalized designing of SACPNs based on unique characteristics of delivered drugs. Specifically, since conventional techniques are mainly conducted in bulk phase, more precisely control over the fabrication process needs further explorations to achieve more accurate regulation of the microstructure, size, and drug distribution of SACPNs. Microfluidics, as the emerging novel technique to precisely manipulate small volume of liquids, can be used as the suitable method for investigating the sophisticated control over the properties of SACPNs since it can increase the control of the entire fabrication process up to an unprecedented level (Ma et al., 2016; Ma et al., 2016; Ma et al., 2016; Deng et al., 2019). Moreover, chemical modifications of chitosan and phospholipid can be used to functionalize SACPNs to broaden its applications. For instance, near-infrared cyanine dyes can be used to modify chitosan. The resultant anticancer drug-loaded SACPNs can therefore generate active singlet oxygen under near-infrared irradiation and can be used as novel vehicles for the combination of chemotherapy and photodynamic therapy (Sharma et al., 2013; Liu et al. 2016; Cao et al., 2018, 2019). Furthermore, personalized design of SACPNs based on unique characteristics of delivered drugs is another major direction. For instance, By investigating the special properties of the delivered drugs and considering their specific application scenarios, SACPNs should be designed to be more versatile to deliver drugs more effectively toward powerful personalized medicines (Corbo et al., 2017, 2018).

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