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Structural lipid nanoparticles self-assembled from electrospun core-shell polymeric nanocomposites†

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Electrospun polymeric core-shell nanocomposites are exploited as templates to manipulate molecular self-assembly for preparing structural lipid nanoparticles, during which the confinement effect of fibers together with their core-shell structure, the aqueous environment and the secondary interactions, all contributed synergistically to push molecular self-aggregation to produce lipid nanoparticles with a drug entrapment efficiency of 95.9% and a sustained drug release profile.

Electrospun nanofibers have been demonstrated to be good templates for indirectly producing functional nano-objects such as inorganic nanotubes, carbon nanofibers and fibrous hydrogel materials with encapsulated microbes.[1-6] These were realized through a strategy that takes the nanofibers as templates in a whole way by virtue of their physical configurations, i.e. through post-treatment of the nanofiber mats (for example by physical absorbance and removing the filament-forming polymer matrix, cross linking reactions, calcinations or carbonization).

Most recently, electrospun monolithic composite nanofibers were demonstrated to be good templates to directly manipulate molecular self-assembly of multiple components for fabricating functional nano-objects *in situ*, such as solid lipid nanoparticles and liposomes.[7,8] In contrast to previous work, these were achieved by virtue of the nanometer confinement effect of the nanofibers and the formulation of a polymeric composite in which the functional building blocks can disperse throughout the polymer matrix on a molecular scale. Similarly, composite microparticles produced by electrospraying were also effective templates for producing self-assembled nanoparticles by virtue of their confinement effect on a microscale.[9]

Although all the nanofiber and composite microparticles have been demonstrated to be good templates in different ways and for different applications, they are products derived from single fluid electrohydrodynamic atomisation (EHDA - electrospraying, electrospinning and e-jet printing [10]) processes which lack secondary microstructure characteristics. Nano-particle self-assembly was achieved mainly through the properties of the components in the nanofibers (often their solubility in special solvents). One of the powerful capabilities of EHDA processes is that they can copy structures from the macro world to products at the micro/nano scale.[11] For example, through the interactions between the electrons and fluid liquid, electrospinning and electrospraying can easily duplicate the structure of macro jet devices (such as concentric, side-by-side and tri-axial spinnerets) to generate products with special microstructures such as core-shell nanofibers/particles, side-by-side and tri-axial nanofibers.[11-14]

Fibers and particles with secondary structure characteristics should be better templates for broader applications in manipulating molecular self-assembly, and designing and developing new advanced materials, than those generated by single fluid processes. Combining two or more fluid EHDA processes can overcome the difficulties of co-dissolving multiple components in a single solvent that often make a single fluid EHDA process a failure. Moreover, through manipulating the spatial distribution of building blocks in the structured fibers or particles, the molecular self-assembly processes may be controlled more accurately and result in higher quality self-assembled products correspondingly.

In this report, we describe the use of core-shell nanofibers produced from coaxial electrospinning as templates for molecular self-assembly to prepare drug-loaded core-shell nanoparticles *in situ*. The hydrophilic polymer polyvinylpyrrolidone K60 was used as the filament-forming matrix, and the lipophilic drug carrier tristearin (GTS) and a poorly water-soluble drug acyclovir (ACY) were used as functional building blocks to demonstrate the strategy.

The poorly water-soluble drug ACY also has poor solubility in a series of typical organic solvents such as ethanol, methanol, chloroform and acetone, but is soluble in N, N-dimethylacetamide (DMAc).[15] PVP has no electrospinnablity in DMAc, thus it is impossible to prepare composite nanofibers of these multiple components using single fluid electrospinning, owing to the lack of cosolubility of the components or good electrospinnability. For a traditional coaxial electrospinning process, the core solution does not need to have electrospinnability, since the shell solution surrounds the core liquid and acts as a guide. [16,17] Here the shell solution is critical and the shell polymer-solvent system selected should be electrospinnable by itself to facilitate formation of a core-shell structure in the fibers. Thus although the core solution consisted of 10% (w/v) PVP and 2% (w/v) ACY in a mixed solvent of DMAc : ethanol (4:6, v:v) and has no electrospinnability, the electrospinnable shell fluid, which consisted of 10% (w/v) PVP and 2% (w/v) GTS in chloroform, can ensure a smooth coaxial electrospinning process and the formation of core-shell fibers with strategic and spatial deposition of building blocks in different parts of the fibers. More details about the co-axial electrospinning process can be found in Supplementary Information.

The field emission scanning electron microscope (FESEM)

images of fibers and their cross-section (Fig. 1 a and b) demonstrate that the fibers had smooth surfaces and a homogeneous inner structure, with no particles separating out from the polymer matrix, either in the shell or in the core parts. Measurement of fiber thickness using Image J software indicated that the fibers have an average diameter of 960 ± 130 nm. Transmission electron microscopy (TEM) images (Fig. 1c) demonstrate the obvious core-shell structure of the fibers, and the uniform gray shading of the shell and core parts of the fibers suggest a homogeneous distribution of GTS and ACY in the PVP matrix in the separate parts of the fibers. Furthermore X-ray diffraction (XRD) patterns (Fig. 1d) indicate that ACY and GTS had lost their original crystalline structures when incorporated into fibers. The results from FESEM, TEM and XRD taken together demonstrate that the components in the composite core-shell fibers were highly mixed and had been converted to an amorphous state.

The distribution of building blocks in the filament-forming polymer matrix on a molecular scale is the first and foremost factor for the fibers to act as templates to direct molecular self-assembly. This ensures that the molecules can be transferred and make contact in a micro-confined region spontaneously when they are liberated in a suitable environment. For electrospun products, polymeric composites can be produced easily by directly exploiting electrical energy to dry and solidify fluid jets containing filament-forming polymer matrix and the guest active ingredient, which produces nano objects very rapidly, often in the order of 10^{-2} s.[18,19] Based on the favorable interactions between the components and the polymer matrix, the physical state of the components in the liquid solutions can be propagated into the solid nanofibers to form a composite.

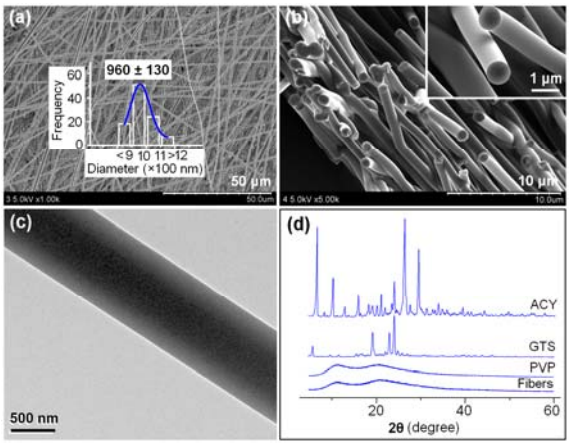

Fig. 1 Characterisation of the electrospun core-shell composite fibers: (a) FESEM image of fibers' surface; (b) FESEM images of fibers' cross-section, the inset has a magnification of ×20,000; (c) TEM images of the fibers' core-shell structure; (d) XRD patterns of ACY, GTS, PVP and fiber composites.

To observe the self-assembly process, a drop of water was placed on fibers collected on a glass slide to initiate the molecular self-assembly process and then was left to dry in air. Shown in Fig. 2a is an image observed using polarization microscopy under cross-polarized light, in which self-assembly events, "frozen" by drying, can be divided into three regions along the water extruding direction indicated by the white arrow. In region 3, there are only swelling fibers. In region 2, there are many bright dots along the fiber lines that appear to be "cut" from the fibers. In region 1, there are many tiny bright dots randomly scattered on the slide. An FESEM image of region 1 is shown in Fig. 2b. The polymer matrix PVP has formed some wrinkles and the self-assembled nanoparticles have separated out from the fiber matrix and dispersed around it. The TEM images of core-shell nanoparticles in Fig. 2c and d demonstrate that the ACY was well encapsulated by GTS, following the template of the core-shell fibers, although there are some GTS nanoparticles without ACY which are greatly smaller than the core-shell nanoparticles.

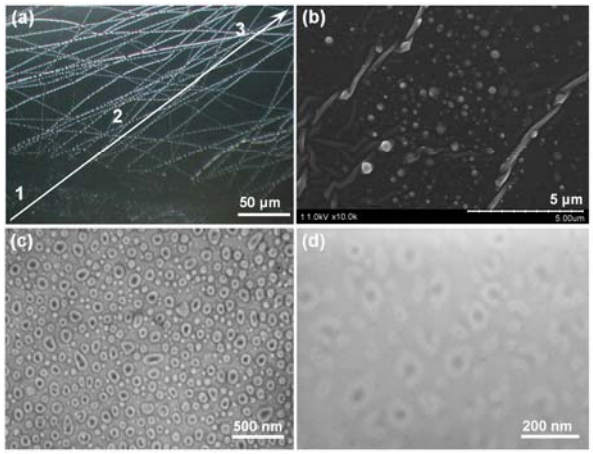

Fig. 2 Self-assembly and characterization of core-shell nanoparticles: (a) Polarization microscopy observation of the self-assembly process occurring when a drop of water was placed on fibers collected on a glass slide (magnification of 7×40); the arrow shows the water extruding direction. (b) FESEM image of a naturally dried area of self-assembly. (c) and (d) TEM images of the nanoparticles under 80 kV for 0.2 s with different magnifications.

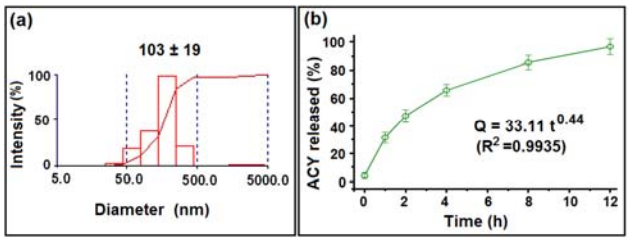

Fig. 3 (a) A typical Static and dynamic light scattering analysis (n=6). (b) The *in vitro* drug release profile (n=6).

A static and dynamic light scattering analysis of the self-assembled lipid nanoparticles showed that they have an average diameter of 103 ± 19 nm (Fig. 3a). During the self-assembly process, a small amount of ACY was also freed into the environmental water. The amount of free ACY in the supernatant from the suspensions was found to be 4.1±2.3%, meaning that 95.9±2.3% of the drug was encapsulated into the structural nanoparticles (Supplementary Information). After 12 h *in vitro* dissolution, 96.6% of the drug in the self-assembled nanoparticles was freed into the dissolution medium (Fig. 3b). According to the Peppas equation:[20] $Q = kt^n$, where Q is the percentage of drug released at time t, k is a

kinetic constant and n is the diffusional exponent indicative of the release mechanism. Drug release from the self-assembled lipid nanoparticles could be fitted with the equation $Q = 33.11t^{0.44}$ (R^2=0.9935). The value of the diffusion index n was 0.44, indicating that ACY release was mainly by a typical Fick diffusion mechanism.

In view of these observations a self-assembly process can be proposed as follows (Fig. 4): 1) The self-assembly process begins with polymer swelling when PVP absorbs water, and the "anchored" building blocks are liberated from the polymer-based composites by water molecules; 2) as the hydrophilic fiber matrix further absorbs water and swells, the compact structure of fibers becomes looser so the building blocks can randomly move in confined regions; 3) the hydrophobic building blocks spontaneously co-aggregate into hybrid "particles" locally due to repulsion forces from the surrounding aqueous environment, and most of the "flexible" PVP molecules that underwent disentanglement are also displaced in the "particles"; 4) the hydrophilic polymer molecules leave the "particle" and dissolve into the dissolution medium, and meanwhile the "particles" condense into nanoparticles. This is why the dots in region 2 are much bigger than those in region 1 in Fig. 2a. The sequence of events is that firstly "particles" are formed when the fibers break up and then form smaller "nanoparticles" through removal of the polymer molecules by water. This suggests that the hydrophobic interactions between the building blocks and the water environment play the key role during the self-assembly process. This, combined with the favorable hydrophobic interactions between the ACY and GTS molecules, allows the transformation of core-shell fibers to core-shell nanoparticles to occur in a controlled, yet spontaneous fashion.

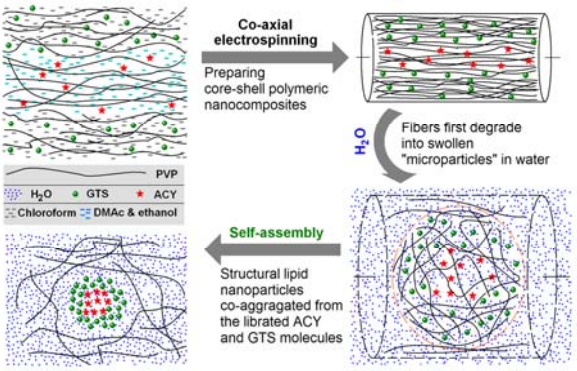

Fig. 4 Process describing the self-assembly of the core/shell nanoparticles from core-shell composite nano-fibers.

When the fibers were put into water, the components in them had been already inherently divided into different kinds according to their solubility in water. The fundamental rules of non-covalent bonding *"Like attracts like"* [21] then can take its role effectively in the confined regions and, based on the fiber structure, push molecular self-assembly. Meanwhile, the properties of the fiber mats (big surface area, high porosity and a continuous web structure) and the highly hygroscopic and hydrophilic properties of the matrix polymer PVP favorably ensure the core-shell fibers spontaneously co-assemble into core-shell nanoparticles.

Self-assembly, defined as the autonomous organization of components into ordered patterns or structures, is able to facilitate the creation of a diverse range of hierarchical nanostructures from a wide range of polymeric and non-polymeric materials.[22] In pharmaceutics, self assembly of different types of small molecules in complex supramolecular structures provides a new way in the development of medicated materials for drug delivery applications (particularly for poorly water soluble drugs).[23] However, the contact of molecules can not be controlled directly on a molecular scale. New methodologies for precisely controlling assemblies of these molecules as building blocks are important.[24, 25] Pre-positioning the building blocks evenly on a polymer matrix to form a nanocomposite can improve our capability to precisely manipulate molecular transport and contact in a confined nano-scale region. Self-assembly based on core-shell nanocomposites, with controlled spatial distributions of components, should be easier and more controllable than traditional methods in which agitations at the macro scale are exploited in attempts to control molecular diffusion and to bring the components into contact. [26] Thus although here we report on the structural lipid nanoparticles self-assembled from core-shell polymeric composites, many other advanced nano drug delivery systems such as micelles, liposomes, nanoemulsions, cubosomes, colloidosomes can be designed and fabricated in a similar way.

On the other hand, the core-shell nanostructure is the most fundamental and popular nanostructure, in which the shell can perform a series of functions such as protection of the core from the outside environment, controlling selective percolation of molecules in and out of the interior of the material and increasing solubility and biocompatibility of drugs. Many complicated nanostructures are essentially the derivatives of this structure, for example by making holes in the shell or encapsulating even smaller nanoparticles in the core. [27] It is often thought that the main methods for generation of core-shell nanoparticles are either bottom-up approaches or top-down approaches, and with the former being more suitable. [28] Presented here is a combined strategy for producing core-shell nanoparticles through transformation at the nanoscale from polymeric core-shell nanocomposites. Electrospinning is developing very quickly in terms of its production scale, [29] new types of processes,[13, 30] applications, [31, 32] and even the new possibility of nanofibers from non-polymeric systems.[33] However, the most fascinating capability of this technology is the generation of core-shell nanofibers. Coaxial electrospinning, [16] modified coaxial electrospinning, [11] emulsion electrospinning, [34] tri-axial electrospinning [13] and also a combined usage of electrospinning with other techniques such as polymerization [35] have been reported for generating core-shell nanofibers. These methods should provide new potential templates for manipulating molecular self-assembly.

Finally, polymers have acted as the backbone for the development of novel DDS during the past several decades. Numerous DDS are prepared through a direct encapsulation of drugs in the polymer matrix and depend solely on the physical-chemical properties of polymers to achieve a desired release profile or drug pharmacokinetics.[36] Most recently, polymer-lipid combined DDS provide a new potential platform for developing

novel DDS.[37, 38] The present study provides a new example of the combined usage of pharmaceutical polymers and lipid. However, in contrast with previous attempts at this combination, in which the polymers are often water insoluble, [37] described here is an investigation of the combined application of hydrophilic polymer with lipid. The core-shell nanocomposites are easy to dispense in water due to the hydrophilic polymer matrix, and the later formed lipid nanoparticles are lipophilic that should facilitate the penetration of drug through bio-membrane. Thus the developed core-shell composites should be particularly useful for poorly water soluble drugs of Class IV for both good dispersion/dissolution and cytomembrane penetrability.[39] Further investigations of these applications are underway.

In summary, a strategy was developed to prepare core-shell lipid nanoparticles through two steps of "copy", i.e. firstly to copy the concentric macrostructure of the spinnerets to produce polymeric core-shell nanocomposites through coaxial electrospinning and subsequently produce core-shell lipid nanoparticles through "copy" by molecular self-assembly based on the core-shell nanofibers. The structure of templates, the components in the core-shell fibers and the surrounding environments acted synergistically to make the self-assembly process accurate and controllable for producing structural lipid nanoparticles *in situ* with a high encapsulation effect and sustained drug release profiles. This approach can be applied to the creation of self-assembled core-shell nanoparticles from a wide variety of materials systems for different types of applications.

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Notes and references

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