Applications of core-shell nanofibers: Drug and biomolecules release and gene therapy

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13.1 Introduction

Electrospinning is a very versatile method for the production of nanofibers and nanofibrous materials, which can be easily functionalized and loaded with a variety of bioactive molecules. Electrospun nanofibers are used as drug delivery systems due to the improvement in the dissolution of the drugs with the increase in the surface area of the materials. In addition, high porosity of the electrospun nanofibrous materials provide efficient loading of bioactive molecules. This technique enables to use both biodegradable and nonbiodegradable polymers and their blends as template for the loading of bioactive molecules and control the release with diffusion and/or erosion of the nanofibrous matrix [1]. The compatibility between bioactive molecules and polymeric matrix chosen for electrospinning is important to control the release of bioactive molecules from the electrospun nanofibrous matrix [2,3]. Yet, even if the bioactive molecules and polymeric matrices are compatible, there are many other factors to be considered for the electrospinning of such systems. For instance, using volatile solvent might cause clogging [4], dissolving the polymers with an organic solvent may cause bioactive molecules to migrate to the surface of nanofibers and therefore burst release of bioactive molecules cannot be avoided [5]. Although electrospinning offers room temperature process for the production of nanofibrous materials and the choice of various kinds of polymers is possible, the necessity of using organic solvents to dissolve most of these polymers creates a major problem related with the stability and bioactivity of the bioactive molecules to be encapsulated in the nanofiber matrix. For instance, the exposure of bioactive molecules especially proteins to harsh condition of organic solvents for a long-time results in denaturation and therefore bioactive molecules could lose their bioactivity. The use of coaxial electrospinning is one of the most promising approaches to avoid the burst release and protect bioactive molecules from organic solvents. During the production of core-shell nanofibers via electrospinning method, two components (core and shell solutions) are fed and then electrospun simultaneously (Fig. 13.1). It is of great importance to adjust the

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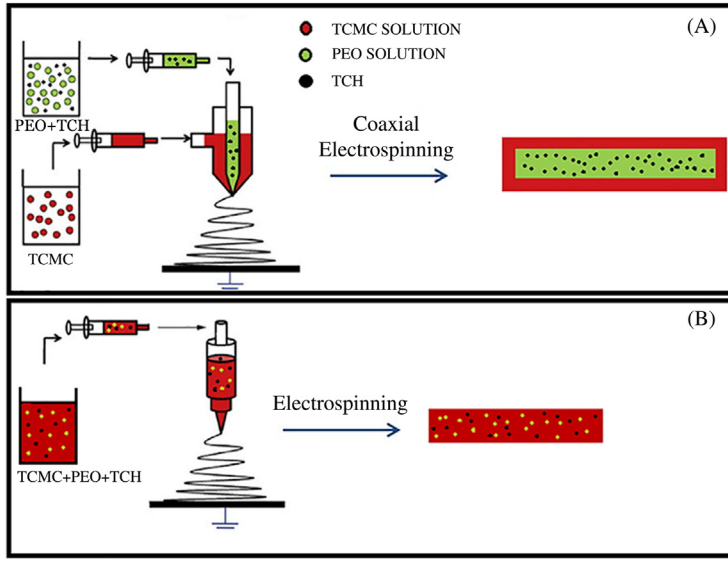

Figure 13.1 Graphical representation of the preparation of nanofibers and drug release (A) coaxial electrospinning; (B) blend electrospinning.
Source: Reprinted with permission from Esmaeili A, Haseli M. Electrospinning of thermoplastic carboxymethyl cellulose/poly (ethylene oxide) nanofibers for use in drug-release systems. Mater Sci Eng C 2017;77:1117−27. Copyright (2017) Elsevier.

additional parameters such as core to shell flow ratio, as well as interfacial tension to obtain nanofibers with core-shell structure and homogenous nanofiber production [6].

Core-shell nanofibers are of great significance to encapsulate various types of bioactive molecules including drugs, proteins, and genes for the sustained release of these molecules due to advantages summarized as follows:

- possibility to obtain nanofibers from un-spinnable solutions [4,8],
- preventing the burst release which might cause toxicological effects [4,9],
- prolonging the time of release and exhibiting sustained release for a longer time [5,10],
- controlling the release kinetics of bioactive molecules by changing the composition [11] or feed rate [12] of core and shell solutions,
- encapsulating the unstable bioactive molecules in mild conditions and protecting biological activity of these molecules [9,13], and
- loading more than one bioactive molecule in the same nanofiber and regulating the rate of release [10,14].

13.2 Delivery of drugs from core-shell nanofibers

13.2.1 Delivery of hydrophilic drugs from core-shell nanofibers

Prolonged release of hydrophilic drugs with the minimum release at the initial stage is a challenge because of their high solubility in aqueous medium. Using both

hydrophobic and hydrophilic polymers cannot be a proper approach for the controlled release of hydrophilic drugs due to incompatibility between drug and hydrophobic polymer causing drug to migrate to the surface of nanofibers [15] and quick solubility of hydrophilic polymers [16]. In addition, producing nanofibers by blending hydrophilic and hydrophobic polymer is a way to reduce the burst release of hydrophilic drugs [16]; however, coaxial nanofibers presents a better approach for the release of hydrophilic drugs in which drugs are encapsulated in the core of the nanofibers.

Core-shell nanofibers can be produced by mixing hydrophilic drugs with polymers in the core [5,7,17,18]. For instance, core-shell and blend nanofibers were produced to encapsulate tetracycline hydrochloride with polyethylene oxide (PEO) in the core and carboxymethyl cellulose in the shell [7]. When core-shell nanofibers are compared with blend nanofibers, burst release was reduced from 54% to 26% and the total release was improved from 76% to 92%. Quick initial release of tetracycline hydrochloride from the blend nanofibers might be due to the hydrophilic polymer (PEO) facilitating water uptake and swelling of the polymeric matrix and the presence tetracycline hydrochloride on the surface of nanofibers [7]. To obtain sustained release, tetracycline hydrochloride was loaded in blend nanofibers of poly (lactic-co-glycolic acid) (PLGA):gum tragacanth (GT) 100:0, PLGA:GT 75:25, PLGA:GT 50:50; and core-shell nanofibers whose core and shell comprised GT and PLGA, respectively [5]. PLGA:GT 50:50 and PLGA:GT 100:0 nanofibers exhibited 48% and 23% burst release and 90% and 35% total release, respectively. In addition, PLGA:GT 50:50, PLGA:GT 75:25, and PLGA:GT 100:0 prolonged the sustained release up to 5, 25, and 7 days, respectively. However, core-shell nanofibers managed to reduce the burst release to 19% and prolonged the sustained release up to 75 days [5]. Similarly, hydrophilic drugs such as ciprofloxacin hydrochloride [17] and tenofovir [18] were incorporated in the core of the nanofibers with a polymer in the core to control the release.

Coaxial electrospinning is of critical importance because it provides electrospinning of nanofibers from unspinnable core [19,20] or shell [21] solutions. For instance, ampicillin was loaded in electrospinnable polycaprolactone (PCL) solution with a partially spinnable PCL solution in the shell [21]. Blend nanofibers released 85% of ampicillin, whereas the initial burst released reduced up to 7% with core-shell nanofibers. Burst release observed in blend nanofibers is most likely due to the lower compatibility between ampicillin and PCL causing ampicillin to the nanofiber surface [21].

A novel approach based on adding graphene oxide (GO) sheets in the core was presented to decelerate the release of a model hydrophilic drug [22]. The model hydrophilic drug, vancomycin hydrochloride was encapsulated in polyvinylpyrrolidone (PVP) blended with GO sheets and PCL was the polymer in the shell [22]. In spite of the existence of GO in the materials design, the nanofibers were biocompatible. In addition, the increasing content of GO in the nanofibers reduced the burst release from 73% to 60%. The improvement was attributed to the molecular interaction between GO and vancomycin hydrochloride. The reduced release rate of vancomycin hydrochloride from nanofibers with increasing amount of GO demonstrated that the amount of release can be adjusted by changing the amount of GO in the nanofibrous material [22].

Another approach to control the drug release with core-shell nanofibers is loading both core and shell with the same drug molecule [12]. Core-shell nanofibers produced with this approach was composed of tetracycline hydrochloride in both core and shell and PLGA in the shell to reduce the diffusion driving force of tetracycline hydrochloride to release [12]. Blend nanofibers possess the higher burst release than core-shell nanofibers. The burst release of core-shell nanofibers with 1%, 0%, and 5% tetracycline hydrochloride in the shell was 44%, 62%, and 73%, respectively. Nevertheless, it was also stated that the shell could not be effective due to the presence of plenty of tetracycline hydrochloride located at the surface of both core and shell layers. Here, another series of nanofibers were also produced to investigate the influence of the flow rate and drug concentration on the release behavior [12]. Increment in the drug concentration was observed to have an increasing effect in the release of nanofibers. In addition, because the amount of encapsulated drug was more in case of higher core flow rate, the burst release of nanofibers produced with higher flow rate of core was much more than that of lower core flow rate. On the other hand, increase in the flow rate of the shell favored less amount of tetracycline hydrochloride release owing to the longer way for drug to go from core to shell and ultimately to the medium [12].

A new strategy to control the release of drugs was based on the production of corks on nanofibers (Fig. 13.2) [23]. To this end, core-shell nanofibers were obtained with silica nanoparticles, which act as corks on the surface of nanofibers [23]. PEO-rhodamine B and polylactic acid (PLA) form the core and shell of the nanofibers, respectively. Although core-shell nanofibers are good at reducing initial burst release of drugs, some amount of burst release was seen because of the defects on the surface of nanofibers. Solvent vapor annealing was utilized to remove surface defects. After annealing, nanofibers has released 0.65%/hour in 24 hours and this release continued as constant for 36 hours, in contrast to 0.9%/hour release of nanofibers without annealing at the initial stage. The release of nanofibers without

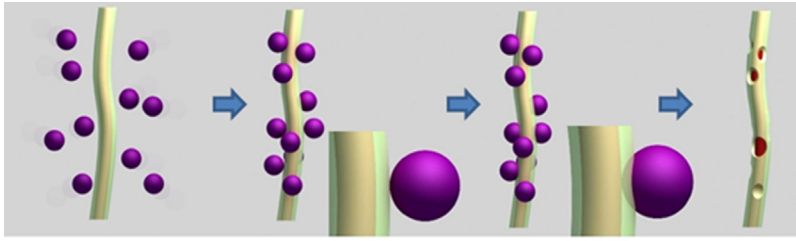

Figure 13.2 Drug release triggered by sonication-induced detachment of clinging nanoparticles. (A) Attachment of nanoparticles onto core-shell nanofibers, (B) embedding of nanoparticles by solvent-vapor annealing, and (C) detachment of nanoparticles by ultrasonication (uncorking) triggering drug release from the core.
Source: Reprinted with permission from Birajdar MS, Lee J. Sonication-triggered zero-order release by uncorking core-shell nanofibers. Chem Eng J 2016;288:1−8. Copyright (2017) Elsevier.

annealing increased up to 2%/hour in 10 hours of time. It was also stated that sonication applied to annealed nanofibers has an enhancing effect on the release amount up to 5%/hour [23].

Cyclodextrins (CDs) are cyclic oligosaccharides which are synthesized by enzymatic degradation of starch. CDs can be employed in many fields including drug delivery due to their capability of making inclusion complex (IC) with a variety of molecules, nontoxicity, and biodegradability. CD was also used to obtain drug-encapsulated core-shell nanofibers (Fig. 13.3) [24]. For this purpose, a hydrophilic drug (propranolol hydrochloride) was incorporated into poly-CD, which was later employed as core and poly (methacrylic acid) (PMAA) as shell solution [24]. Finally, nanofibers were annealed at 170°C for 48 hours to enhance the hydrophobicity of the material. Blend nanofibers released 30%−35% of propranolol hydrochloride at the first 8 hours, in contrast 15% of release from core-shell nanofibers. As regards to total release, blend and core-shell nanofibers released 40% and 23% of propranolol hydrochloride in 168 hours. Here, cross-linking of poly-CD and

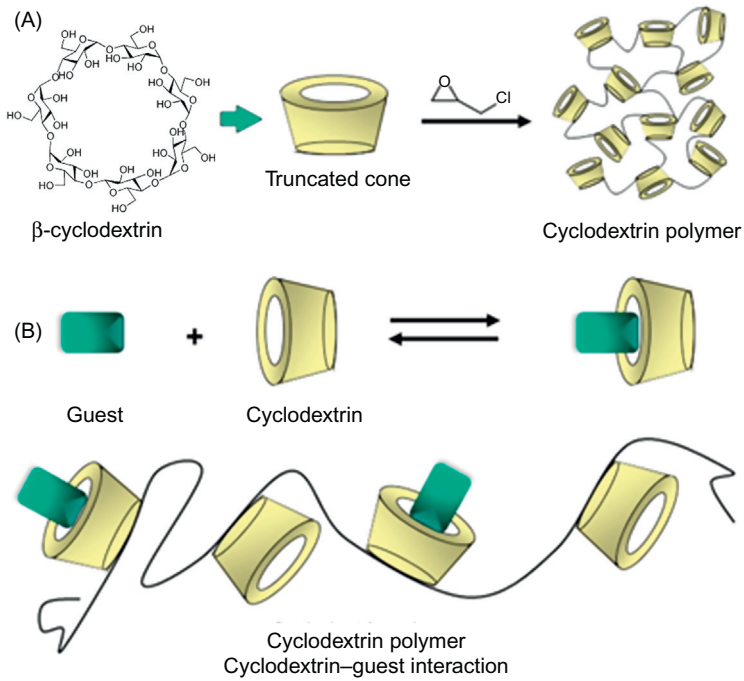

Figure 13.3 (A) Chemical structure of β-CD and a schematic representation of its truncated cone-shape and the reaction with epichlorohydrin to produce a CD-based polymer network and (B) comparison between CD and poly-CD host−guest interactions.
Source: Reprinted with permission from Oliveira MF, Suarez D, Rocha JCB, de Carvalho Teixeira AVN, Cortés ME, De Sousa FB, et al. Electrospun nanofibers of polyCD/PMAA polymers and their potential application as drug delivery system. Mater Sci Eng C 2015; 54:252−61. Copyright (2017) Elsevier.

PMAA, and interaction of propranolol hydrochloride with poly-CD were the major factors contributing to the retarded release of propranolol hydrochloride from core-shell nanofibers [24].

13.2.2 Delivery of hydrophobic drugs from core-shell nanofibers

Hydrophobicity of some of the drugs is a common problem encountered in pharmaceutical industry. Loading drugs in electrospun nanofibers is known to have an improving effect on the solubility of these drugs. On the other hand, it is also of importance for hydrophobic drugs to be released in a sustained manner. The compatibility between drug and carrier polymeric matrix is quite important route to achieve sustained release [15]. However, many studies showed that core-shell nanofibers are effective to control the release of hydrophobic drugs as well.

Core-shell nanofibers of hydrophobic drugs were achieved by loading drugs in the core of the nanofibers with a polymer [11,25–27]. Paclitaxel (PTX)-loaded PCL/polyurethane (PU) nanofibers-coated stent was designed via electrospinning (Fig. 13.4) [11]. By keeping the total drug content same (0.5%, w/v), the amount of drug in the core of the nanofibers was reduced; in contrast, the amount of drug in the shell was increased. Four nanofibers produced by this method were different from each other in terms of release behavior. Thus, total PTX release was the highest when the amount of PTX was 0.35% in the shell due to the easy diffusion of drug from the shell. However, the rate of PTX release was lower when the amount of PTX in the core (0.35%) was more than the shell [11].

In another example, an anticancer drug, doxorubicin was incorporated in the core of core-shell nanofibers consisting of polyvinyl alcohol (PVA) core and chitosan shell via electrospinning technique [25]. To prevent doxorubicin leakage from dissolution into the medium quickly, nanofibers were cross-linked with glutaraldehyde vapor for 3 minutes. It was deduced from the release results that release rate can be adjusted by changing the feed rate of the shell solution. When the flow rate of the shell increased, the initial release reduced from 22% to 13% in 2 hours of time. However, total release of doxorubicin decreased from 68% to 43% when the flow rate increased [25]. Similar results in which acyclovir [26] and flurbiprofen axetil [27] were inserted in the core with a hydrophilic polymer were also obtained.

Coaxial electrospinning enables to produce nanofibers from unspinnable core or shell solutions [4,28–30]. For instance, core-shell nanofibers were produced by using spinnable zein solution with ketoprofen in the core and unspinnable zein (1%) solution in the shell [4]. In contrast to nanofibers of single electrospinning (45% initial release), core-shell nanofibers released only 5%–10% of ketoprofen, thus, these core-shell nanofibers did not exhibit a burst release. Therefore, thin layer of zein in the outer part of the nanofibers prevented the diffusion and quick burst release of ketoprofen from core-shell nanofibers. In addition, total release of blend nanofibers were completed at the concentration of 93% in 8 hours, whereas core-shell nanofibers released 92% of ketoprofen in 16 hours. So, sustained release was

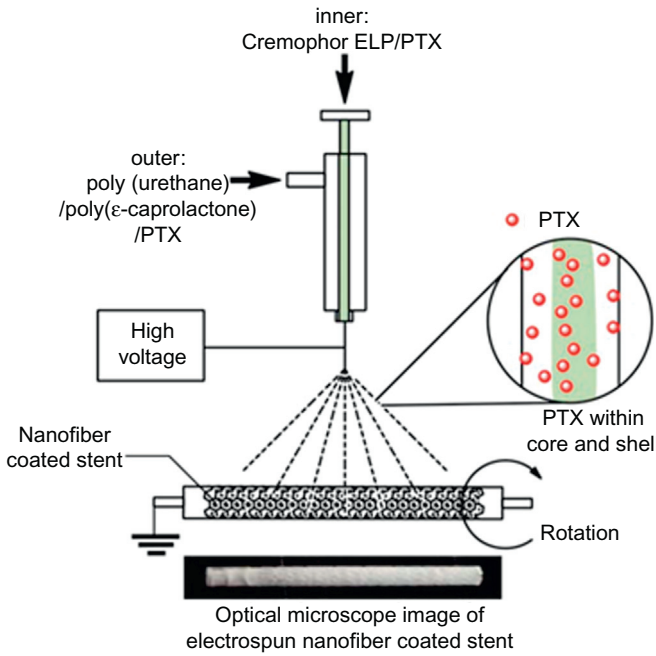

Figure 13.4 Schematic illustration of preparing nanofiber-coated stents for controlled release of PTX. The shell (PCL/PU/PTX) and the core (PTX /Cremophor) were co-electrospun through a dual nozzle onto the slowly rotating bare metal stent to prepare nanofiber-coated drug-eluting stents. Optical microscopic image of the fiber-coated stent is shown in the bottom. Through the coaxial electrospinning, different amount of PTX is incorporated in both the shell and the core to manipulate drug release profiles.
Source: Reprinted with permission from Son YJ, Kim HS, Choi DH, Yoo HS. Multilayered electrospun fibrous meshes for restenosis-suppressing metallic stents. J Biomed Mater Res Part B Appl Biomater 2017;105(3):628−35. Copyright (2017) Wiley.

also achieved with core-shell nanofibers. In addition to diffusion retarding effect, zein layer on the outer of the core was also important to prevent clogging of the spinneret [4]. Core-shell nanofibers was similarly achieved by using only a solvent in the shell for more uniform nanofibers and controlled release of drugs such as ibuprofen [28] and ketoprofen [29]. Likewise, dexamethasone solution, which is unspinnable with a single electrospinning, was loaded without a polymer in the core of nanofibers [30].

CD-ICs provide enhancement in the solubility of hydrophobic molecules owing to the host−guest interaction formed between CD and drugs in appropriate polarity and dimension. There are several studies in the literature aiming to improve the solubility and controlling the release of numerous molecules by incorporating CD-IC of molecules such as vanillin [31], eugenol [32], allyl isothiocyanate [33], sulfisoxazole [34], quercetin [35], gallic acid [36], α-tocopherol [37,38], and thymol [39] into polymeric

solutions and ultimately nanofibers were produced via electrospinning. Moreover, there are also studies in which CDs was used as template and host simultaneously for the IC of triclosan [40], geraniol [41], vanillin [42], limonene [43], sulfisoxazole [44], and linalool [45]. Starting from this point of view, core-shell nanofibers in which the core and shell comprised CD-IC of a model hydrophobic compound and polymer was designed [46]. CD-IC of curcumin forms the core, whereas PLA forms the shell of the nanofibers (Fig. 13.5) [46]. PLA nanofibers with free curcumin were produced as a reference sample (PLA−curcumin nanofibers). Both of the nanofibers released much more amount of curcumin at pH 1 compared to pH 7.4 which was likely due to the increased solubility of curcumin at pH 1. Core-shell nanofibers had a slower rate of curcumin release at both conditions owing to the presence of shell in their structure increasing the time of curcumin to reach the medium. In addition, total amount of curcumin released from core-shell nanofibers was more than PLA−curcumin nanofibers. This is most likely due to the solubility enhancement of curcumin as shown in the phase solubility test [46].

A novel strategy was proposed to control the release of hydrophobic drugs by using oppositely charged nanospheres to increase the interaction with the drug [47]. Here, vancomycin and oppositely charged gelatin nanospheres were incorporated into silk fibroin/PEO nanofibers by using electrospinning technique [47]. It was concluded that nanofibers with vancomycin-loaded gelatin nanospheres were much more successful for the delivery of vancomycin in a sustained manner more than 14 days. Nevertheless, nanofibers without nanospheres could not release vancomycin after 2 days of release experiment. The sustained release of core-shell nanofibers with gelatin nanospheres might be ascribed to the attractive interaction between vancomycin and gelatin nanospheres [47].

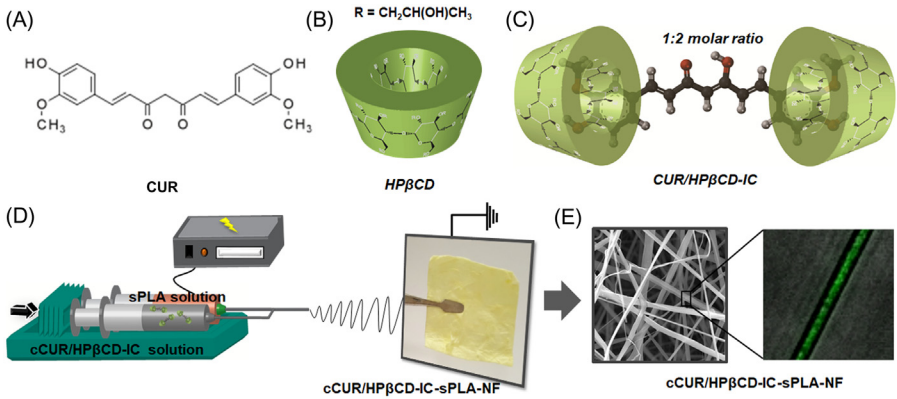

Figure 13.5 (A) Chemical structure of curcumin (CUR); schematic representation of (B) HPβCD, (C) formation of CUR/HPβCD-IC, (D) electrospinning of core-shell nanofibers from cCUR/HPβCD-IC-sPLA solution, (E) TEM and CLSM images of cCUR/HPβCD-IC-sPLA-NF.

13.2.3 Delivery of drugs from fast-dissolving core-shell nanofibers

Fast-dissolving delivery systems are of significance for especially pediatric and geriatric patients due to the difficulty of these patients in the swallowing of the medicines [48]. Electrospinning could be a simple and versatile alternative approach to produce fast-dissolving nanofibers containing drugs. However, sometimes it is hard to find the suitable solvent for the active agent to facilitate the electrospinnability of the system in a single electrospinning system. Therefore, core-shell nanofibers could be a more efficient way to produce fast-dissolving nanofibers.

Nanofibers with core-shell structure might be obtained with a fast-dissolving character by using hydrophilic polymers in both core and shell [49,50]. For instance, fast-disintegrating drug delivery system of a hydrophobic flavonoid, quercetin, was produced via coaxial electrospinning [49]. PVP was used both in core and shell of the nanofibers; sodium dodecyl sulfate (SDS) was also added to the shell solution. Many factors including hygroscopicity and hydrophilicity of PVP, three-dimensional structure of nanowebs increasing the surface area of the nanofibers, surface tension reducing effect of SDS facilitating the electrospinnability and enhancing the wettability of nanofibers, contributed to the dissolution of quercetin with PVP. Core-shell nanofibers released quercetin in 1 minute and had distinct release profiles as compared to quercetin powder. Therefore, they are good candidates as oral fast-disintegrating drug delivery system [49]. Core-shell nanofiber, which is a new type of acid−base pair solid dispersion, was produced to enhance the dissolution rate of an acidic drug [50]. Quercetin, PVP, and sodium hydroxide were the components of the core fluid, whereas citric acid and PVP form the shell fluid. However, in addition to the high surface area and porous structure of nanofibers, hydrophilicity of PVP core-shell nanofibers released the entire drug in 1 minute [50].

In a similar approach drug can be encapsulated both in the core and in the shell [51]. Core-shell nanofibers composed of quercetin−ethyl cellulose (EC) core and quercetin−PVP shell exhibited sustained release for 24 hours [51]. In addition, the amount of burst release which is due to the quercetin in the shell increased with the increasing drug content in the nanofibers [51].

Core-shell nanofibers were used for not only drug release but also for bitter taste masking [52]. Fast-disintegrating core-shell nanofibers were developed which can provide rapid oral delivery of helicid and mask the unpleasant taste of the formulation via coaxial electrospinning [52]. Sucralose and PVP were added to the shell composition of the nanofibers in which helicid was located in the core with PVP. Core-shell nanofibers released entire helicid in 1 minute owing to the 3D structure, high surface area, amorphous physical state of drug, and hydrophilic polymer (PVP) in the structure. In contrast, commercially available helicid dispersible tablets released 89% of helicid in 30 minutes. Furthermore, developed nanofibers were also effective to mask the bitter taste of the drug by the release sweetener, sucralose loaded in the shell of nanofibers [52].

13.2.4 Delivery of drugs by electrospun nanofibers from triaxial systems

Triaxial electrospinning is also important for drug delivery. Due to the presence of an intermediate layer between core and shell, this system is of importance for delivering more than one bioactive molecule from the same nanofiber. Triaxial nanofibers could possess different levels of hydrophobicity and mechanical strength as well. However, because of the complexity of the system, there are only a few studies on nanofibers produced by triaxial system [53].

Triaxial electrospinning is useful in encapsulating more than one bioactive molecule [54]. In a study, two kind of model dye molecules (keyacid blue and keyacid uranine) were incorporated in different parts of the coaxial and triaxial nanofibers (Fig. 13.6) [54]. Coaxial nanofibers were composed of PVP and keyacid blue in the core and PCL (c-PVP-keyacid blue/s-PCL nanofibers) or PCL and keyacid uranine (c-PVP-keyacid blue/s-PCL-keyacid uranine nanofibers) in the shell. Triaxial

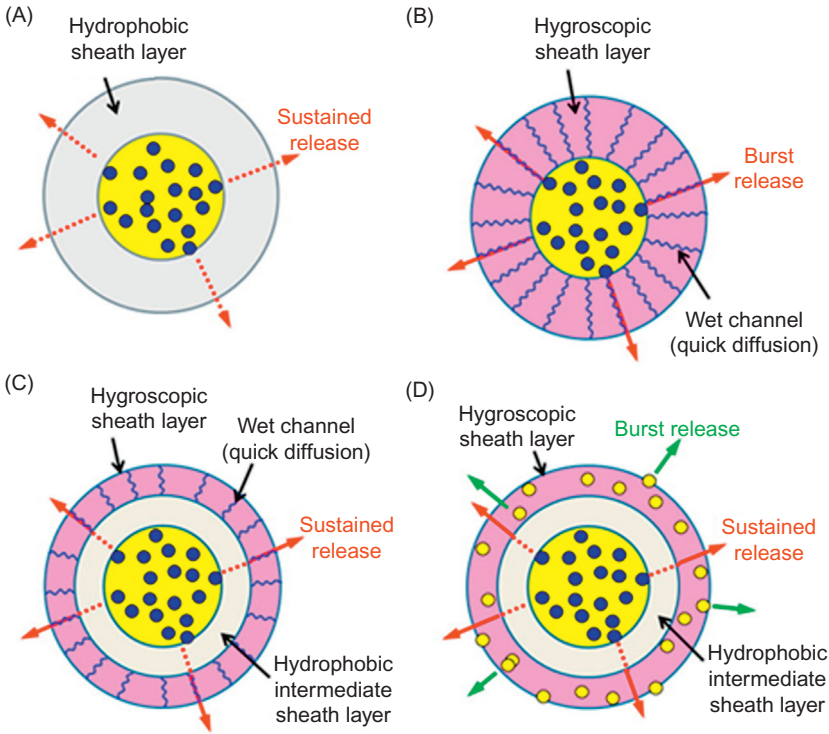

Figure 13.6 Cross-section of coaxial and triaxial fibers: (A) coaxial fiber with hydrophobic sheath, (B) coaxial fiber with hygroscopic sheath, (C) triaxial fiber with hygroscopic sheath, and (D) triaxial fiber loaded with dual drugs.
Source: Reprinted with permission from Han D, Steckl AJ. Triaxial electrospun nanofiber membranes for controlled dual release of functional molecules. ACS Appl Mater Interfaces 2013;5(16):8241−5. Copyright (2017) American Chemical Society.

nanofibers comprised PVP-keyacid blue, PCL, PCL-keyacid uranine as core, intermediate, and shell layers, respectively (t-c-PVP-keyacid blue/i-PCL/s-PCL-keyacid uranine nanofibers). The sustained release profile was obtained when keyacid blue was released from c-PVP-keyacid blue/s-PCL nanofibers. If another drug is loaded as in the case of c-PVP-keyacid blue/s-PCL-keyacid uranine nanofibers, significant burst release with a minimum sustained release was observed. It was explained that hygroscopic sheath, which absorbs water and forms channels, resulted in the burst release from core. Triaxial nanofibers, which have an intermediate layer between core and shell, have overcome the problem of c-PVP-keyacid blue/s-PCL-keyacid uranine nanofibers. For instance, triaxial nanofibers released 80% of keyacid blue in 24 hours, whereas c-PVP-keyacid blue/s-PCL-keyacid uranine nanofibers released the same amount of keyacid blue in 1 hour. Additionally, the effect of the thickness of the core nozzle and flow rate of the shell on the release were investigated. Release results of keyacid blue showed that the rate of release decreases with increasing flow rate of shell and decreasing core nozzle diameter having thicker intermediate layer. Lastly, keyacid uranine, which was located in the outermost layer of triaxial nanofibers exhibited abrupt burst release in all samples [54].

13.3 Delivery of proteins from core-shell nanofibers

Encapsulation of proteins into the polymeric nanofibers via electrospinning is a simple alternative approach. However, single electrospinning is not good enough at inhibition of proteins from denaturation due to the long time direct exposure of protein with the harsh organic solvents [55,56]. In addition, blending the proteins in the polymeric solution might cause an agglomeration close to the surface of nanofibers which later results in the burst release of proteins [57]. Thereby, coaxial electrospinning emerges as a modified technique to protect sensitive molecules like proteins and control their release.

Through coaxial electrospinning it is possible to produce nanofibers from a core, which is composed of only proteins [58]. In a study, core-shell nanofibers was produced with poly (L-lactic-ε-caprolactone) [P(LLA-CL) 50:50] as shell and a protein, bovine serum albumin (BSA) as core [58]. The release of BSA from core-shell nanofibers was investigated for 14 days, and it was observed that BSA released in a sustained manner after 25%−30% initial burst release, which was most likely caused by BSA located on the surface of nanofibers [58].

Emulsion electrospinning was also applied to produce core-shell nanofibers encapsulating proteins [59]. Core-shell nanofibers were achieved via emulsion electrospinning by using BSA and polystyrene (PS) in the core and shell, respectively [59]. While preparing nanofibers, four types of PS having different molecular weight were dissolved in L-limonene, which is a green solvent. A modest burst release (17%−41%) of BSA was observed from nanofibers for 2 days; afterwards sustainability of BSA release from nanofibers for 50 days was varied depending on the molecular weight of PS used. Thus, nanofibers with the lowest molecular

weight of PS exhibited enhanced sustained release compared to other nanofibers. This result might be related to the distribution of BSA, inner structure of nanofiber, and porosity of fiber shell matrix. Higher molecular weight reduces the evaporation rate of solvent and solidification of the fiber occurs slowly, and because of these reasons, BSA is located closer to the surface. In addition, increment of molecular weight weakens the intermolecular entanglements and leads to enhanced steric hindrance of polymer backbone, and ultimately this factor causes an increase in the porosity degree of fiber wall and matrix. Therefore, BSA diffused easily through the matrix of nanofibers with higher molecular weight [59].

Core-shell nanofibers composed of polymer and protein in the core and polymer in the shell was also reported [60,61]. BSA was incorporated in the core part of core-shell nanofibers with the help of polyethylene glycol (PEG) to stabilize protein and increase the viscosity of the solution [60]. In this case, BSA was labeled with fluorescein isothiocyanate and the shell polymer was PCL. It was seen that the initial burst release was more severe (60%−70%) in case of blend nanofibers in contrast to 45%−65% release of core-shell nanofibers. SEM images taken after the release experiment showed that blend nanofibers has very rough and eroded-like structure with obvious pits and/or cavities; conversely, core-shell nanofibers only became flatter and collapsed compared with the previous cylindrical shape [60]. In another study about encapsulation of BSA in core-shell nanofibers, two types of core-shell nanofibers (c-BSA-PEG/s-PCL nanofibers and c-BSA/s-PCL nanofibers) were obtained in addition to the nanofiber produced by blend electrospinning (b-BSA-PEG-PCL nanofibers) [61]. Confirmation of core-shell structure was done by transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM), and images demonstrated the homogenous distribution of BSA in c-BSA-PEG/s-PCL nanofibers. Burst release occurred from blend and core-shell nanofibers; however, core-shell nanofibers exhibited more sustained release behavior after this step, because core-shell−structured nanofibers act as a protein reservoir and also a barrier membrane controlling the protein diffusion rate. According to the Ritger and Peppas equation, n values of c-BSA-PEG/s-PCL nanofibers and blend nanofibers were calculated to be $\sim$0.45 and $\sim$0.37 indicating that release kinetic was diffusion-based and irregular protein transportation related, respectively. Moreover, c-BSA-PEG/s-PCL nanofibers have higher diffusion as compared to c-BSA/s-PCL nanofibers, but they have advantage of higher protein activity. Hence, PEG including system was better at preservation of the activity of protein most likely due to reduced protein adsorption to the organic polymer phase during electrospinning [61].

13.4 Delivery of enzymes from core-shell nanofibers

Enzymes are protein molecules, which are mostly hydrophilic and catalyze the reactions in the body. However, structural instability like all proteins is the main issue encountered with the enzymes. Electrospinning is a commonly used method to

immobilize several types of bioactive agent such as drugs, growth factors, and genes owing to the high surface to volume ratio enabling quite high loading and possibility to produce nanofibers at room temperature. Core-shell nanofibers obtained via electrospinning technique is a good alternative, because incorporating enzymes in the core of the nanofibers protects them from denaturation, which might be caused by the harsh solvent medium. Therefore, enzymes loaded in the core-shell nanofibers could preserve their bioactivity and prolong the release [55,56].

Core-shell nanofibers to encapsulate enzymes were produced with different approaches. In a first approach, enzyme solution which is unspinnable was used as a core with a hydrophilic [62] or hydrophobic [63] shell. Lactate dehydrogenase is an enzyme encapsulated in the core part of the nanofibers whose shell is composed of PVA (c-lactate dehydrogenase/s-PVA nanofibers) [62]. In addition, core-shell nanofibers in which the core was PVA and the shell was lactate dehydrogenase was produced as well (c-PVA/s-lactate dehydrogenase nanofibers). The absorbance increment observed was due to the conversion of nicotinamide adenine dinucleotide ($NAD+$) to dihydronicotinamide adenine dinucleotide (NADH) with the help of lactate dehydrogenase. C-lactate dehydrogenase/s-PVA nanofibers exhibited more sustained release than c-PVA/s-lactate dehydrogenase nanofibers. However, the burst release seen in the first 2 days in c-lactate dehydrogenase/s-PVA nanofibers might be due to the diffusion of enzyme molecules to the surface of the nanofibers during electrospinning process because of the immediate swelling of PVA, which is a hydrophilic polymer, in the release medium. On the other hand, c-lactate dehydrogenase/s-PVA nanofibers cross-linked in concentrated methanol solution possessed much more sustained released behavior than non-cross-linked ones owing to the increase in the water resistance of PVA nanofibers [62]. Coaxial electrospinning was also employed to immobilize multienzyme systems involving cofactor regeneration *in situ* [63]. In this case the shell solution was prepared by dissolving PU in dimethylacetamide at 30% (w/v) of polymer concentration. Coenzymes with a molecular weight lower than 20 kDa were released quickly (90% of NADH released in 5 minutes.), but enzymes including 3α-hydroxysteroid dehydrogenase and diaphorase did not exhibit any detectable release due to their molecular weight higher than 20 kDa, because hollow nanofiber wall had a characteristic molecular weight cutoff at approximately 20 kDa [63].

In the second approach, polymer is also added to the core solution [13]. Core-shell nanofibers of PVA (shell) and gelatin (core) was used to load lysozyme in the core to decelerate its release rate [13]. Cross-linked core-shell nanofibers and blend nanofibers were also produced as reference. Cross-linked core-shell nanofibers were shown to have the slowest rate of release of enzyme as compared to both non-cross-linked core-shell and blend nanofibers [13].

A last interesting example is related to a core solution composed of enzymes encapsulated in liposomes, whereas polymer solution forms the shell of the nanofibers [64]. The main purpose of this study was primarily to enhance delivery efficiency and preserve the activity of the chosen enzyme, horseradish peroxidase [64]. Fluorescein isothiocyanate−dextran was incorporated into the core as the monitoring fluorescent probe. PVA and PCL were the polymers used in the core and shell,

respectively. It was seen that nanofibers without liposome released 60% of horseradish peroxidase, whereas nanofibers with liposome released only 20% of horseradish peroxidase in 24 hours. Furthermore, the half time of these systems was determined to be 20 and 112 hours, respectively. It was also confirmed that liposome-containing nanofibers were good at preserving the enzymatic activity due to the shielding effect of the lipid sphere [64].

13.5 Delivery of growth factors from core-shell nanofibers

There exist a number of biological stimuli, which are ubiquitously used to promote cell proliferation and differentiation. Among these, growth factors (GFs) are a significant class, which transmit required signals to control cell proliferation, differentiation, and extracellular matrix secretion. GFs have short plasma half-life (usually several minutes) and rapid degradation *in vivo*, and as a result, they require repeated and local administration at large doses to be clinically effective. In addition, GFs are usually hydrophilic, quite low amount of GFs are enough, and excess amount might cause side effects. Therefore, it is of great importance for GFs to be released in a controlled manner. Direct injection of these kinds of bioactive molecules is not effective because of the rapid diffusion from the target site or deactivation by the enzymes *in vivo* [65–67]. As a result, coaxial electrospinning is a well-known approach, which provides sustained release from the scaffold by maintaining the bioactivity of GFs.

Core-shell nanofibers whose core do not include polymer were also produced [68–70]. Electrospinning was employed to encapsulate fibroblast growth factor (FGF) which is known to be involved in tissue regeneration and mesenchymal stem cell proliferation and differentiation in PLGA nanofibers [68]. Blend and coaxial electrospinning were the two techniques used to produce FGF encapsulated nanofibers. It was revealed from the release test that nanofibers produced via coaxial electrospinning released FGF during 14 days in a sustained manner, whereas nanofibers obtained from blend electrospinning could release FGF only 7 days [68]. Core-shell nanofibers with aligned morphology were fabricated for peripheral nerve regeneration by using PLGA in the shell and nerve growth factor (NGF) in the core of the nanofiber [69]. Sustained release of NGF from the core of aligned core-shell nanofibers continued for 30 days. Approximately 30% of NGF was released in the first day, and then nanofibers released NGF in a relatively steady manner for the following 29 days [69]. Similar approach was also used to encapsulate bone morphogenetic protein 2 in core-shell nanofibers [70].

Emulsion electrospinning is known as a better alternative to reduce the initial burst release of drugs or proteins and protect the bioactivity of incorporated drugs or proteins [71,72]. Vascular endothelial growth factor (VEGF) was loaded in the core of nanofibers by emulsion electrospinning with a protective agent, either dextran or BSA (Fig. 13.7) [71]. The release of VEGF from nanofibers was

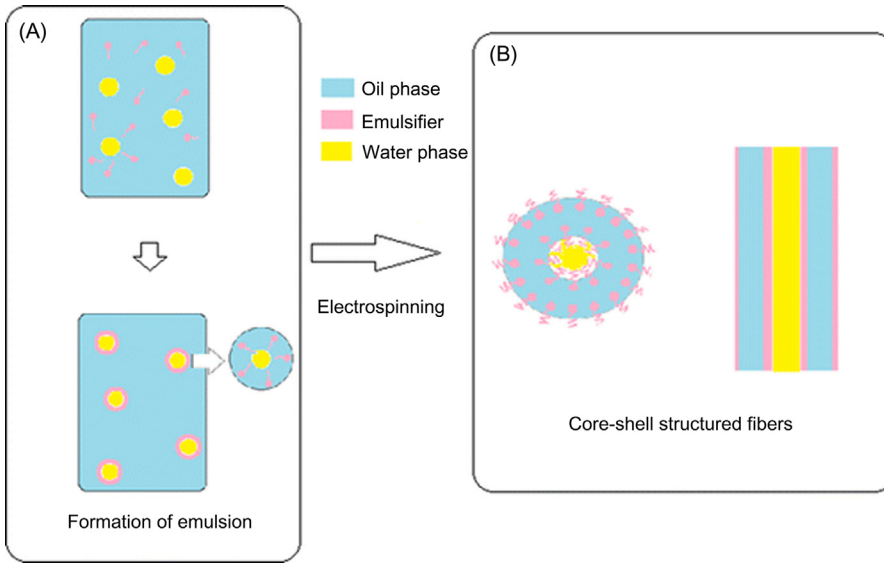

Figure 13.7 Schematic illustration of the (A) formation of emulsion and (B) core-shell structured nanofibers produced by electrospinning.
Source: Reprinted with permission from Tian L, Prabhakaran MP, Ding X, Kai D, Ramakrishna S. Emulsion electrospun vascular endothelial growth factor encapsulated poly (l-lactic acid-co-ε-caprolactone) nanofibers for sustained release in cardiac tissue engineering. J Mater Sci 2012;47(7):3272−81. Copyright (2017) Springer.

investigated for 28 days. Though the second stage of c-VEGF-BSA/s-poly(L-lactic acid-co-ε-caprolactone) (PLCL) and c-VEGF-dextran-s-PLCL nanofibers were very similar to each other, initial release of the nanofibers were quite different. Thus, c-VEGF-BSA/s-PLCL nanofibers released almost 10%, whereas c-VEGF-dextran/s-PLCL nanofibers released only 1% of VEGF in 24 hours. The higher release amount of c-VEGF-BSA-/s-PLCL nanofibers might be associated with the VEGF that was possibly located at the surface of nanofibers [71]. Epidermal growth factor (EGF) was also incorporated into core-shell nanofibers with the emulsion electrospinning approach to control its release [72]. Core-shell nanofibers produced with polymeric core and shell were also used to encapsulate growth factors like NGF [73] and EGF [74].

13.6 Core-shell nanofibers for gene therapy

Gene delivery induces production of GFs or other related proteins by the cells *in situ*. In contrast to viral vectors protecting genes from degrading enzymes, that are infectious, expensive, hard to prepare, and have low loading of genes, nonviral vector including liposomes or polymers like chitosan produce less antigenicity,

lower transduction efficiency, and shorter expression duration [67]. Gene delivery with scaffolds loaded with genes has been proposed as a new approach, which is considered as an effective delivery carrier. Electrospun nanofibers are good candidates for gene delivery due to the high surface area and porous structure but direct contact of genes with the organic solvents for long time during electrospinning most likely results in loss of genes activity. Therefore, coaxial electrospinning is the preferred technique to deliver genes [57,67].

Core-shell nanofibers utilized for gene delivery can be produced by a hydrophilic polymer in the core and a hydrophobic polymer in the shell [75–77]. Core-shell nanofibers were designed with plasmid DNA within the core and the nonviral gene delivery vector poly(ethylenimine)-hyaluronic acid (PEI-HA) within the shell of the nanofibers [75]. PEG and PCL were the polymers used in the core and shell, respectively. The release of PEI-HA was investigated depending on PCL concentration, PEG concentration, molecular weight of PEG, and concentration of plasmid DNA. Burst release was between 9% and 47% in 24 hours, whereas the cumulative release was between 35% and 144% in 60 days of release. However, the release rates for the different systems were not significantly different from each other after 24 hours of release. It was also seen that the main factor influencing the release was the concentration of plasmid DNA [75]. In a similar manner, virus was encapsulated in the core of PCL nanofibers with PEG to release the viral vector in a porogen-mediated mechanism [76]. In another study, scaffolds in the form of core-shell nanofibers were designed from bioactive PEI/plasmid BMP2 (pBMP2) and PLGA [77]. Blend and core-shell nanofibers exhibited 42% and 32% of pBMP2 release at the initial stage, respectively. Sustained release of pBMP2 was also achieved during 28 days from core-shell nanofibers [77].

13.7 Stimuli-responsive core-shell nanofibers for delivery of biomolecules

13.7.1 pH triggered delivery of biomolecules

Using pH sensitive polymers is a common method to obtain targeted release of bioactive molecules. Eudragit L100-55, Eudragit L100, and Eudragit S100 are known to be dissolved above pH 5.5, 6.0, and 7.0, respectively. Since Eudragit methacrylate polymers are insoluble in highly acidic medium and soluble in basic medium, they are widely used to coat the tablets for a colon-targeted delivery of bioactive molecules [78].

First, bioactive molecules were incorporated in the core of the nanofibers with an accompanying polymer. In this approach, pH sensitive Eudragit polymer was placed either in the core or shell of the nanofibers [79–81]. Core-shell nanofibers were developed comprising of relatively high content of helicid and Eudragit L100-55 in the core, helicid, and PVP in the shell [79]. Blend nanofibers composed of only core solution (helicid-Eudragit L100-55 nanofibers) and shell solution (helicid-PVP

nanofibers) were also produced as references. To investigate the release behavior of nanofibers in the body, nanofibers were first placed in gastric fluid, which has a pH of 2 for 2 hours. Then, nanofibers were moved to pH 7 to mimic intestinal fluid for 8 hours. As expected, helicid-PVP nanofibers released 100% of helicid in 1 hours in acidic medium because of the quick dissolution of the PVP. Owing to the insoluble nature of Eudragit L100-55 in acidic medium, helicid-Eudragit L100-55 nanofibers released only 3% of helicid during 2 hours in acidic medium. Core-shell nanofibers released 54% of helicid in 2 hours, and remaining helicid were released in a sustained manner for 6 hours to the basic medium. However, it was deduced that released amount of drug in different phases can be changed by the concentrations of drug in core and shell solutions and flow ratio of core to shell during the electrospinning process [79]. Core-shell nanofibers were produced comprising Eudragit EPO [poly(butyl methacrylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate)] and tetracycline (core) and Eudragit L100-55 [poly(methacrylic acid-co-methyl methacrylate)] (shell) [80]. Three types of nanofibers were produced by using 40:60, 50:50, and 70:30 EPO:L100-55. 40:60, 50:50, and 70:30 EPO:L100-55 released 15%, 23%, and 62% of tetracycline in 5 minutes, respectively. However, release of 70:30 EPO: L100-55 was reduced at pH 6, whereas 50:50 EPO:L100-55 released much more tetracycline at pH 6. This result agreed well with the dissolution test in which 70:30 EPO:L100-55 dissolved better at pH 2 than pH 6, whereas 50:50 EPO:L100-55 dissolved better in pH 6 than pH 2. It was also seen that electrostatic interaction between tetracycline and Eudragit L100-55 is more pronounced when the amount of negatively charged Eudragit L100-55 increased in the system, thus from 70:30 EPO: L100-55 to 50:50 EPO:L100-55. Therefore, the least amount of tetracycline released from 40:60 EPO:L100-55 reflected the effect of interaction on the controlling the release of tetracycline from nanofibers. Furthermore, the remaining tetracycline in the nanofibers before and after release at pH 2 and pH 6 were visualized with fluorescence images (Fig. 13.8). The images of 40:60 and 50:50 EPO:L100-55 exhibited higher fluorescence intensity at pH 2 compared to pH 6 due to the low amount of release in this medium. The images of 70:30 EPO:L100-55 after released at pH 2 and 6 were not much different from each other because most of the tetracycline released at pH 2 from this nanofiber [80].

An anticancer drug, 5-fluorouracil, was encapsulated into the core of the nanofibers alone (c-5-fluorouracil/s-Eudragit S100 nanofibers), with PVP (c-5-fluorouracil-PVP/s-Eudragit S100 nanofibers), and Eudragit S100 (c-5-fluorouracil-Eudragit S100/s-EudragitS100 nanofibers) [81]. To be used as a reference, 5-fluorouracil loaded blend Eudragit S100 nanofibers were also produced (5-fluorouracil-Eudragit S100 nanofibers). Even though Eudragit S100 shell is not soluble in acidic medium, it was not capable of preventing the release in the acidic medium. Blend and core-shell nanofibers released 5-fluorouracil quickly in both acidic and basic medium. High solubility of 5-fluorouracil in acidic medium and its low molecular weight facilitating the release from the pores might be the reasons for high amount of 5-fluorouracil release from blend nanofibers into acidic medium. Although the presence of clear interface between core and shell compartments and insoluble polymer in the shell, the reason of core-shell nanofibers to release 5-fluorouracil rapidly in

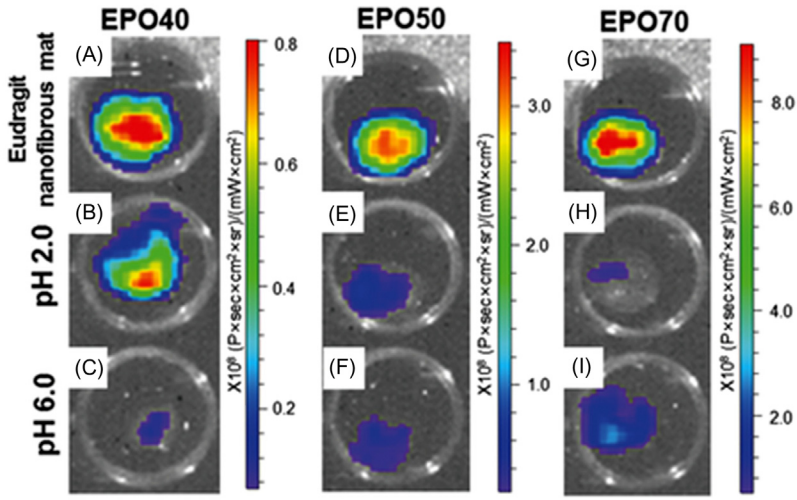

Figure 13.8 Fluorescence images of tetracycline in Eudragit nanofibrous mats after electrospinning (A, D, and G) and after soaking of the Eudragit nanofibrous mat at pH 2.0 (B, E, and H) and at pH 6.0 (C, F, and I), respectively, for 10 minutes. Eudragit nanofibrous mats were washed with distilled water to remove released tetracycline. Fluorescence images of the mat were obtained by *in vivo* imaging system to visualize the fluorescence of tetracycline and subsequently superimposed with light images of the mats in the multiwell plates ($\lambda ex = 410-440$ nm, $\lambda ex = 445-490$ nm).
Source: Reprinted with permission from Son YJ, Kim Y, Kim WJ, Jeong SY, Yoo HS. Antibacterial nanofibrous mats composed of eudragit for pH-dependent dissolution. J Pharm Sci 2015;104(8):2611−8. Copyright (2017) Wiley.

pH 1 could be some mixing of core and shell solutions and the observed broken structure in the nanofibers after immersing them. Nevertheless the dramatic release of core-shell nanofibers in the pH 1.0 medium, the nanofibers have shown to exhibit two-stage release profiles. Therefore, there was some 5-fluorouracil in the nanofibers to be released in gastrointestinal tract, and this amount could be manipulated by playing with the polymer composition of the core. On the other hand, c5-fluorouracil-PVP/s-Eudragit S100 nanofibers exhibited sustained release as compared to other nanofibers, and this is related with the hydrophilicity of PVP in the nanofiber structure. Because water reaches the core through the pores in Eudragit S100, it causes PVP to swell and the nanofibers to burst, losing the integrity. Formation of agglomerate might explain the sustained release of this core-shell nanofiber [81].

There exist studies in which a bioactive molecule was in the core with a pH sensitive polymer and shell of core-shell nanofibers were employed to prevent clogging of the core solution [82,83]. In one of the studies, diclofenac sodium was encapsulated in Eudragit L100 which later forms the core of nanofibers, whereas unspinnable solvent mixture of ethanol and N,N-dimethylacetamide (DMAc) was the shell solution [82]. Core-shell nanofibers obtained with this method provide

better distribution of diclofenac sodium and better colon-targeted release with a longer sustained period. The release of core-shell and blend nanofibers were investigated in acidic medium (pH 1.5) for 2 hours, then the medium was changed to basic (pH 6.8, 5 hours) to mimic stomach and colon, respectively. Of note, 5% release of diclofenac sodium in 2 hours into acidic medium might be due to the diclofenac sodium located on the surface of nanofibers. However, when pH was changed to 6.8, both blend and core-shell nanofibers released diclofenac sodium due to the dissolution of the polymer in basic medium, thus core-shell nanofibers released 41% in total, whereas blend nanofibers released 56%. This result makes core-shell nanofibers better in terms of sustained and targeted release in colon. In addition, the physical shape of the nanofibers also had an effect on the release behavior of diclofenac sodium from each nanofiber. Despite the fact that blend nanofibers were thicker than core-shell nanofibers, the longest diffusion distance of blend nanofibers were smaller than the diameter of core-shell nanofibers because of the flat morphology of the blend nanofibers. Drug release mechanism showed that exponent values of 0.98 and 0.84 for blend and core-shell nanofibers and the release of nanofibers were based on non-Fickian diffusion mechanism in which both diffusion and erosion play a role in the release of diclofenac sodium from nanofibers [82]. In another study, ferulic acid was incorporated into the shellac which is in the core of the nanofibers with a shell comprised solvent mixture (Fig. 13.9) [83]. As expected, core-shell nanofibers did not release ferulic acid much in pH 2 for 2 hours that is to mimic stomach conditions. When nanofibers were transferred to basic medium with a pH of 6.8, they released 10% of ferulic acid loaded in the nanofibers in 30 minutes of immersion. This result is the confirmation of the better sustained release

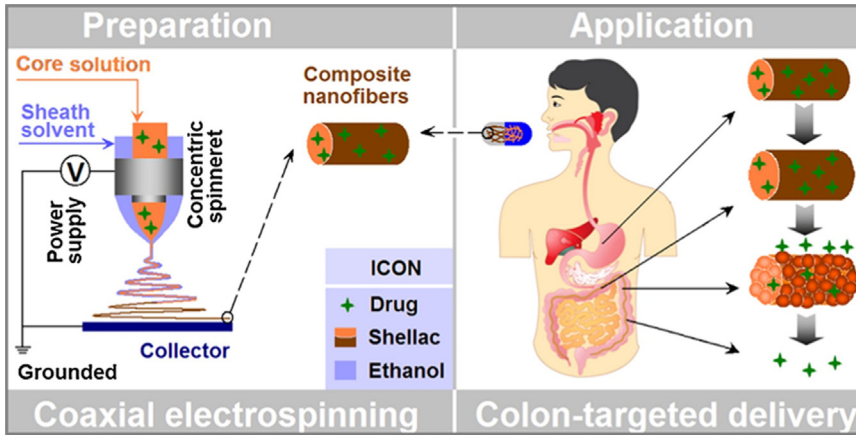

Figure 13.9 A schematic illustrating the strategy underlying the design of the medicated shellac nanofibers prepared in this work.
Source: Reprinted with permission from Wang X, Yu DG, Li XY, Bligh SA, Williams GR. Electrospun medicated shellac nanofibers for colon-targeted drug delivery. Int J Pharm 2015;490(1):384−90. Copyright (2017) Elsevier.

achieved with core-shell nanofibers, because it is known that 40% of pure ferulic acid is released in 30 minutes in pH 6.8. SEM images of nanofibers were also taken to observe the release related morphology change. Because dissolution of shellac resulted in nanofibers to be curved and broken, their diameter to be raised, and most importantly nanoparticles to appear, it was concluded that the erosion mechanism of shellac is different from the erosion mechanism of other polymers. Therefore, erosion-controlled mechanism of designed core-shell nanofibers in basic medium is suitable for colon-targeted delivery of drugs [83].

Triaxial system was applied to yield pH sensitive core-shell nanofibers, which is produced from three different solutions. Among these solutions, core is composed of bioactive molecule, intermediate solution is a pH sensitive polymer solution, and the outermost solution is the solvent [8]. Here, pH-sensitive Eudragit S100 nanofibers were loaded with lecithin and diclofenac sodium in the core for colon-targeted delivery (Fig. 13.10) [8]. Ethanol was used in the outer part of the nanofibers. Another importance of this study is to show the production of core-shell nanofibers from one (Eudragit S100 solution) of the three fluids being electrospinnable. Because diclofenac sodium and Eudragit S100 are insoluble in acidic condition, triaxial nanofibers released only 2% of diclofenac sodium in 2 hours, after transferring the nanofiber to the neutral medium and 79% of diclofenac sodium was released in 22 hours. Here, the release of diclofenac sodium from nanofibers occurred in two successive steps at neutral pH, because the dissolution of Eudragit S100 lecithin−diclofenac sodium core was converted into particles and some of diclofenac sodium released into the medium. Then, the remaining diclofenac sodium is gradually released from the particles to the medium via diffusion [8].

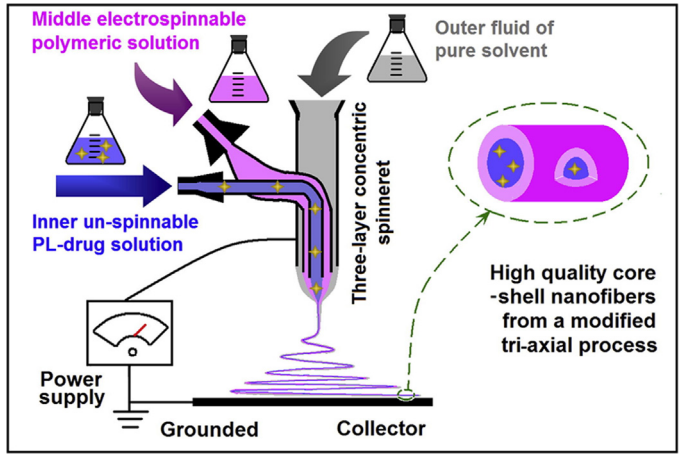

Figure 13.10 A diagram of the modified triaxial electrospinning process and its use for preparing core-shell drug-loaded nanofibers.
Source: Reprinted with permission from Yang C, Yu DG, Pan D, Liu XK, Wang X, Bligh SA, et al. Electrospun pH-sensitive core−shell polymer nanocomposites fabricated using a tri-axial process. Acta Biomater 2016;35:77−86. Copyright (2017) Elsevier.

13.7.2 Delivery of biomolecules from thermoresponsive core-shell nanofibers

Stimuli-responsive polymers are polymers which are able to respond to the changes in the environment such as pH and temperature. Poly(N-isopropylacrylamide) (PNIPAAm) is one of most commonly used of those polymers responsive to temperature. Thus, PNIPAAm is soluble in water below its lower critical solution temperature (LCST, 31°C−35°C) and precipitates above its LCST [84].

In an approach aiming to produce thermoresponsive core-shell nanofibers, biomolecules were loaded in the core with a thermoresponsive polymer [85]. Thermoresponsive coaxial nanofibers were developed by using ketoprofen and PNIPAAm in the core and EC in the sheath (c-PNIPAAm-ketoprofen/s-EC nanofibers) [85]. It was seen that coaxial nanofibers were quite good at exhibiting both sustained and thermoresponsive release. PNIPAAm nanofibers with ketoprofen (PNIPAAm-ketoprofen nanofibers) and blend nanofibers with EC and ketoprofen (b-PNIPAAm/EC-ketoprofen nanofibers) were also produced as controls. The highest and the lowest released amount at 25°C were observed from PNIPAAm-ketoprofen nanofibers and c-PNIPAAm-ketoprofen/s-EC nanofibers, respectively. The possible reason for the difference in the release of these nanofibers could be the variance in the surface wettability of nanofibers. B-PNIPAAm-EC-ketoprofen nanofibers and c-PNIPAAm-ketoprofen/s-EC nanofibers exhibited more sustained released as compared to PNIPAAm/ketoprofen nanofibers due to the EC in the structure increasing the hydrophilicity of the systems. As regards to 37°C, c-PNIPAAm-ketoprofen/s-EC nanofibers showed a sustained release for 55 hours with a burst release of around 25% in the first 5 hours owing to the EC in the shell of the nanofiber. Much greater release was seen at 25°C than at 37°C from all nanofibers. But c-PNIPAAm-ketoprofen/s-EC nanofibers were able to reduce this effect and combined thermoresponsive feature of PNIPAAm and sustained release ability of coaxial nanofibers. When the temperature increases from 25°C to 37°C, EC, which is in the sheath, tighten up and core nanofiber shrinks and in addition when the temperature goes back to 25°C nanofibers restore the original state [85].

In another approach, thermoresponsive core-shell nanofiber was produced by the combination of UV-photo-polymerization and electrospinning [86]. To this end, PLA loaded with a model drug combretastatin A4 was electrospun first, then N-isopropylacrylamide (NIPAM) was coated as a shell layer on the nanofibers by UV photopolymerization (c-PLA-combretastatin A4/s-PNIPAAm nanofibers) [86]. First, it was seen that the wettability property of the nanofibers changed with the temperature, thus when the applied temperature is below the LCST, the nanofibers were hydrophilic, whereas when the applied temperature is above the LCST nanofibers, they were hydrophobic. Because of the hydrophilic nature of PNIPAAm at the temperature lower than LCST (i.e., 25°C), the release medium could reach to the core part of c-PLA-combretastatin A4/s-PNIPAAm nanofibers. However, due to the hydrophobic feature of PLA, 50%−60% of combretastatin A4 remained in the core of the nanofibers. On the other hand, when the temperature reaches to 40°C, which is a temperature higher than the LCST, as seen in the wettability

results, PNIPAAm turns into a hydrophobic polymer and due to this transition the shell deformation occurs and 70% of the drug is released from the nanofibers. Moreover, it was also seen that drug release rate reduced after UV exposure and with the increasing time of UV exposure, and drug release was inhibited because of the shell thickness. However, the release rate can be controlled by keeping the time of exposure at a desired level [86].

13.8 Delivery of multiple drug and biomolecules from core-shell nanofibers

Coaxial electrospinning is a widely used simple approach to release multiple bioactive molecules. In a first approach, two types of bioactive molecules can be loaded in the core of the nanofibers [9,10,87–89]. Platelet-derived growth factor (PDGF) and BSA were incorporated in the core of nanofibers whose shell was composed of PCL and PEG [10]. PEG was used as a porogen at different molecular weight (3400 and 8000 g/mol) and concentration (1 and 20 mg/mL) in the shell, thus to increase fiber swelling and pore formation. Although the location of BSA in the core of nanofibers was quite effective to obtain sustained release (50% release in 40 days), the presence of PEG improved the BSA release in a concentration and molecular weight–dependent manner. The fastest release was observed when PEG molecular weight is 3400 g/mol and the highest release was achieved when the concentration is 20 mg/mL. Therefore, increasing PEG molecular weight lowers the release rate and the concentration has an increasing effect in the total release. As regards to PDGF, 100% release was observed in 35 days from nanofibers having PEG, with a relatively linear profile but nanofibers without PEG could not release enough amount of PDGF (less than 1%) in 35 days. In addition, encapsulation efficiency, which was nearly 100%, showed that coaxial electrospinning was not only effective in sustained release but also in loading ability for GFs and other type of proteins [10]. In another study, core-shell nanofibers were composed of BSA and NGF in the core and PLLACL in the shell [9]. In this case, the release of BSA from blend nanofibers exhibited quick burst release; however, core-shell nanofibers released only 10%–20% of BSA at the initial step. In addition, encapsulation efficiency of BSA has reached up to 93% in case of core-shell nanofibers. Finally, it was concluded that NGF released from the core-shell nanofibers retained bioactivity up to 10 days [9]. Core-shell nanofibers were designed by incorporating BMP2 and insulin-like growth factor 1 (IGF1) at different flow ratio [87]. BSA was a stabilizer in the system. The release result of the nanofibers showed that nanofibers with higher shell flow ratio released BMP2, IGF1, and BSA more slowly in 3 days and in a more sustained manner up to 28 days of release [87]. Similar studies evaluated the release of BSA and NGF [9], BSA and tetrapeptide val-gal-pro-gly [88], and antibiotics (vancomycin and ceftazidime) and BMP2 from core-shell nanofibers [89].

The effect of the location of two different bioactive molecules in the core-shell nanofibers was investigated as well [14,90]. BMP2 and dexamethasone were incorporated in PLLACL/collagen nanofibers by blending (b-BMP2-dexamethasone-PLLACL-collagen nanofibers) or coaxial electrospinning (c-BMP2/s-dexamethasone-PLLACL-collagen nanofibers and c-BMP2-dexamethasone/s-PLLACL-collagen nanofibers) [14]. BSA was also used for stabilization. More controlled release of protein and drug was observed with the core-shell nanofibers as compared to the blend nanofibers. Blend nanofibers released 44%−49% and 71%−81% of dexamethasone and BSA at the initial stage and in total, respectively. The burst release of BSA and dexamethasone reduced up to 17%−19% when BSA and dexamethasone were located in the core of the nanofibers, whereas almost 45% of dexamethasone and 20% of BSA released from core-shell nanofibers in which dexamethasone was in the shell and BSA was in the core. The total amount of dexamethasone released was 71%, 75%, and 61% and from blend nanofibers, c-BMP2/s-dexamethasone-PLLACL-collagen nanofibers, and c-BMP2-dexamethasone/s-PLLACL-collagen nanofibers, respectively. Of note, 81%, 64%, and 74% of BSA was released from blend nanofibers, c-BMP2/s-dexamethasone-PLLACL-collagen nanofibers, and c-BMP2-dexamethasone/s-PLLACL-collagen nanofibers, respectively. As a result, sustained release of dexamethasone and BSA was achieved for 22 days from core-shell nanofibers, especially with c-BMP2-dexamethasone/s-PLLACL-collagen nanofibers [14]. Three types of core-shell nanofibers with PLLACL were produced: BSA located in the core and rhodamine B was in the shell (c-BSA/s-PLLACL-rhodamine B nanofibers), rhodamine B located in the core and BSA was in the shell (c-rhodamine B/s-PLLACL-BSA nanofibers), and both BSA and rhodamine B were in the core and PLLACL was in the shell (c-BSA-rhodamine B/s-PLLACL nanofibers) [90]. Blend nanofibers released 53%−67%, whereas c-BSA-rhodamine B/s-PLLACL nanofibers released only 12%−18% of both BSA and rhodamine B in the first 6 hours. As expected c-BSA/s-PLLACL-rhodamine B nanofibers and c-rhodamine B/s-PLLACL-BSA nanofibers released rhodamine B and BSA fast, respectively. So, shell structure in c-BSA-rhodamine B/s-PLLACL nanofibers serves as an extra layer for the slow release of molecules [90].

Another approach is adding hydrophilic polymers like PEG or PVA to the core solution in addition to the biomolecules [91,92]. In a study, the controlled release of two types of protein was investigated separately [91]. BSA or lysozyme was encapsulated in the core with PEG by using PCL in the shell of nanofibers. Initially, slight burst release was observed during the first day and this was followed by sustained release. The thickness of the inner part was adjusted by changing the feed rate of the core solution, and it was deduced that higher feed rate resulted in more rapid protein release. Additionally, both proteins were intact; hence, they were protected from denaturation by core-shell structured nanofibers [91]. In a different study, PLGA/collagen nanofibrous scaffolds incorporated with fibronectin and cadherin 11 were designed as a tissue engineering material [92]. The loading of the proteins were done in the core of the nanofibers with PVA. 25% and 80% of fibronectin/cadherin 11 was released at the end of 1 day and 14 days, respectively. Therefore, in addition

to slight burst release of fibronectin/cadherin 11 from core-shell nanofibers, nanofibers also showed a controlled release by the time. The released amount of fibronectin/cadherin 11 stated to be enough to promote the proliferation and differentiation of human mesenchymal stem cells at different stages [92].

In another approach, one of the bioactive molecules in the core was encapsulated in nanospheres [93]. For instance, core-shell nanofibers were produced to sequentially deliver dual growth factors, FGF18 and FGF2 from core of the nanofibers for bone regeneration [93]. PEO and PCL were the polymers used for this system in core and shell, respectively. Mesoporous bioactive glass nanospheres (MBNs) were employed as nanocarrier for the encapsulation of FGF2 in one of these nanofibers. Cytochrome C was used as a model drug instead of FGF2 to evaluate the release behavior of the nanofibers. Outer shell layer composed of PCL, acting as the diffusion barrier, inhibited the quick release from both nanofibers. When the release behavior and rate from core-shell nanofibers with MBNs and without MBNs are compared, MBN-loaded nanofibers delayed release to a later point in time. But the release pattern of the nanofibers was similar and the initial release was not so much different from the nanofibers without MBNs [93].

13.9 Conclusion

Core-shell nanofibers produced via electrospinning technique were shown to be an effective strategy for controlled release of bioactive molecules including drugs, proteins, and genes. The main advantages of this approach are making possible to obtain nanofibers from unspinnable solutions; homogenous distribution of the bioactive molecules; in addition to mitigation of the burst release, prolonged sustained release. There exist various methods to obtain controlled release with this type of nanofibers other than loading the bioactive molecules in the core of the nanofiber. Addition of bioactive molecules in the shell as well, playing with the concentration of the bioactive molecules in the core and shell, cross-linking the system, addition of nanospheres, using several compounds in the core to increase the interaction with the bioactive molecules and thus to delay the release of bioactive molecules. Triaxial nanofibers with an intermediate polymer layer were also developed to further decelerate the release of bioactive molecules. Core-shell nanofibers were also found to be superior in terms of preserving bioactivity of the bioactive molecules loaded in the core alone or with other means such as liposomes, against exposure of solvents for long time. Moreover, core-shell nanofibers are especially useful for loading multiple bioactive molecules to manipulate the release rate of several bioactive molecules. Similarly, loading sweeteners to the shell of the nanofibers is an alternative approach to mask the bitter taste of the drugs loaded in the core. Additionally, stimuli-responsive core-shell nanofibers were designed as well, and among these, core-shell nanofibers produced with pH-sensitive polymers are of great importance for efficient targeted release of bioactive molecules.

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