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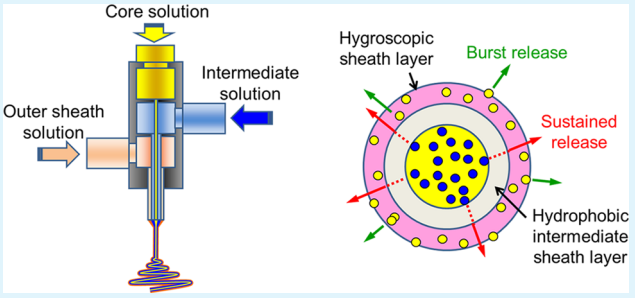
Triaxial Electrospun Nanofiber Membranes for Controlled Dual Release of Functional Molecules

Daewoo Han and Andrew J. Steckl*

Nanoelectronics Laboratory, University of Cincinnati, Cincinnati, Ohio 45221-0030, United States

Supporting Information

ABSTRACT: A novel dual drug delivery system is presented using triaxial structured nanofibers, which provides different release profiles for model drugs separately loaded in either the sheath or the core of the fiber. Homogenous, coaxial and triaxial fibers containing a combination of materials (PCL, polycaprolactone; PVP, polyvinylpyrrolidone) were fabricated. The drug release profiles were simulated using two color dyes (KAB, keyacid blue; KAU, keyacid uranine), whose release in physiological solution was measured using optical absorption as a function of time. To reach the level of 80% release of encapsulated dye from core, triaxial fibers with a PCL intermediate layer exhibited a ~24× slower release than that from coaxial fibers. At the same time, the hygroscopic sheath layer of the triaxial fibers provided an initial burst release (~ 80% within an hour) of a second dye as high as that from conventional single and coaxial fibers. The triaxial fiber membrane provides both a quick release from the outer sheath layer for short-term treatment and a sustained release from the fiber core for long-term treatment. The intermediate layer between inner core and outer sheath acts as a barrier to prevent leaching from the core, which can be especially important when the membranes are used in wet application. The formation of tri/multiaxially electrospun nanofibrous membranes will be greatly beneficial for biomedical applications by enabling different release profiles of two different drugs from a membrane.



KEYWORDS: *electrospinning, triaxial structure, nanofiber, dual release, controlled delivery*

INTRODUCTION

Most common methods for drug/protein delivery (e.g., oral ingestion, injection) to a targeted site lead to undesired side effects due to delivery to untargeted sites and the introduction of high doses of the drug in order to reach the intended target. Therefore, encapsulation of functional molecules, such as drugs, proteins, and genes, has been intensively studied for targeted and controlled delivery.[1,2] Encapsulation has the potential to serve a dual role by controlling the release profile and protecting the therapeutic agent from the ambient environment.

Nanofiber membranes containing drug/protein molecules are an attractive approach for localized delivery of drugs to a targeted site.[3−7] Drug loaded electrospun fibers produced by single nozzle electrospinning have been reported earlier,[8] but the loaded drug experienced an initial burst release followed by rapid decay due to the fact that the functional molecules on the fiber surface are directly exposed to the ambient environment. To solve this problem, core−sheath structured fibers have been investigated for the controlled release of drugs/proteins. Coaxial electrospinning provides a versatile one-step method to produce core−sheath structured fibers with excellent control of the fiber membrane properties (e.g., fiber composition, morphologies, etc.),[9−12] which is beneficial for controlling drug release characteristics. The encapsulated drug/protein in the fiber core can be released through the sheath layer in a controlled manner, lasting significantly longer (from several hours to months)[13−15] than conventional fiber membranes[3] (Figure 1a).

In general, controlled release from molecules encapsulated in the core of coaxial fibers is only possible when the fiber sheath material is nonhygroscopic. When the sheath layer is hygroscopic by either incorporating water-soluble molecules or using hygroscopic polymers, water molecules form channels between the fiber core and the outer environment, as illustrated in Figure 1b. Therefore, release from the core occurs fairly rapidly, with characteristics closer to burst release rather than controlled release. This is a problem in many in vivo applications because some materials (e.g., gelatin, collagen, peptides, etc.) are preferred for the sheath layer due to their excellent biocompatibility and are frequently also hygroscopic.

To solve this issue, we have investigated three-layer (core/intermediate/sheath) structured fibers produced by triaxial electrospinning.[16−19] As shown in Figure 1c, the intermediate layer acts as a buffer region between the inner core and the outer sheath. The triaxial fiber approach is particularly important when using hygroscopic material for the sheath in order to obtain excellent biocompatibility. In this case, the

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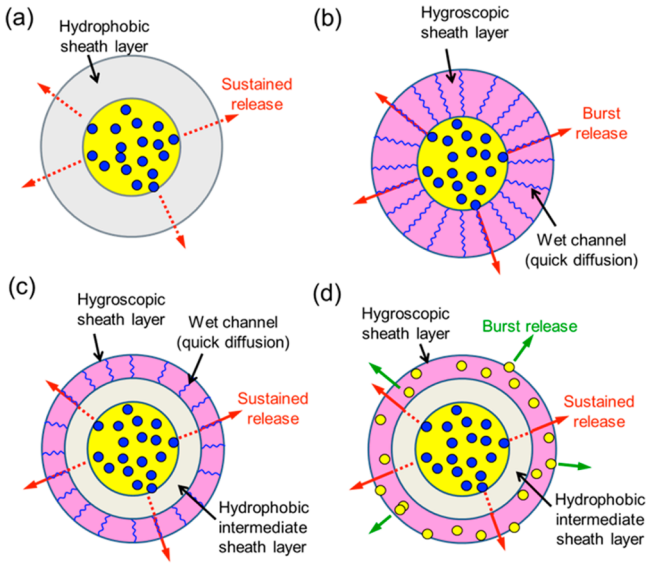

Figure 1. . Cross-section of coaxial and triaxial fibers: (a) coaxial fiber with hydrophobic sheath; (b) coaxial fiber with hygroscopic sheath; (c) triaxial fiber with hygroscopic sheath; (d) triaxial fiber loaded with dual drugs.

hydrophobic intermediate layer forces the functional molecules from the core to diffuse through the intermediate layer, resulting in sustained release rather than being rapidly released by dissolution.

Novel delivery vehicles are being investigated[20−25] for the combined delivery of multiple drug molecules with different release time profiles. Multiaxial fibers produced by electrospinning are very promising for providing a versatile multidrug delivery vehicle. In addition to the sustained release from the fiber core for long-term treatment, various functional molecules can be incorporated into the sheath layer for short-term treatment. This dual delivery system not only provides two different drugs with different release profiles but also enables synergistic effects such as combined drug and gene therapy[23] to improve the healing process. Selective loading of multiple functional molecules with different release profiles is a challenging issue in conventional encapsulation, but can easily be accomplished in a single step by using the multiaxial electrospinning method. Triaxial fibers are particularly attractive because the intermediate layer can act as an encapsulation layer between core and outer sheath (Figure 1d). This allows for the loading of water-soluble drug/protein molecules in the sheath layer without causing a premature (burst) release from the molecules encapsulated in the core.

EXPERIMENTAL SECTION

Electrospinning. The electrospinning technique including multiaxial electrospinning is a very versatile tool to produce nanofibrous membranes made of various natural and/or synthetic materials, including biopolymers, liquid crystalline polymers, textile fiber polymers, electrically conducting polymers, etc.[26,27] A very fine liquid jet is ejected under high electric field from a liquid droplet at the tip of the nozzle. The highly charged liquid jet experiences bending and stretching effects due to charge repulsion and, in the process, becomes continuously thinner, down to the nanometer range. During the bending and whipping action experienced by the liquid jet, the volatile solvent is thoroughly evaporated and the resulting solidified nanofibers are collected on the conducting substrate, as illustrated in Figure 2a.

The coaxial electrospinning apparatus at the University of Cincinnati has been previously described.[28,29] For triaxial electrospinning experiments, triaxial nozzles purchased from NanoNC (Seoul, South Korea) and a third syringe pump for the intermediate solution have been added to the coaxial electrospinning setup. The triaxial nozzle illustrated in Figure 2b has inner diameters of core, intermediate, sheath of 0.3, 0.85, 1.6 mm and outer diameters of 0.55, 1.25, 2.1 mm, respectively.

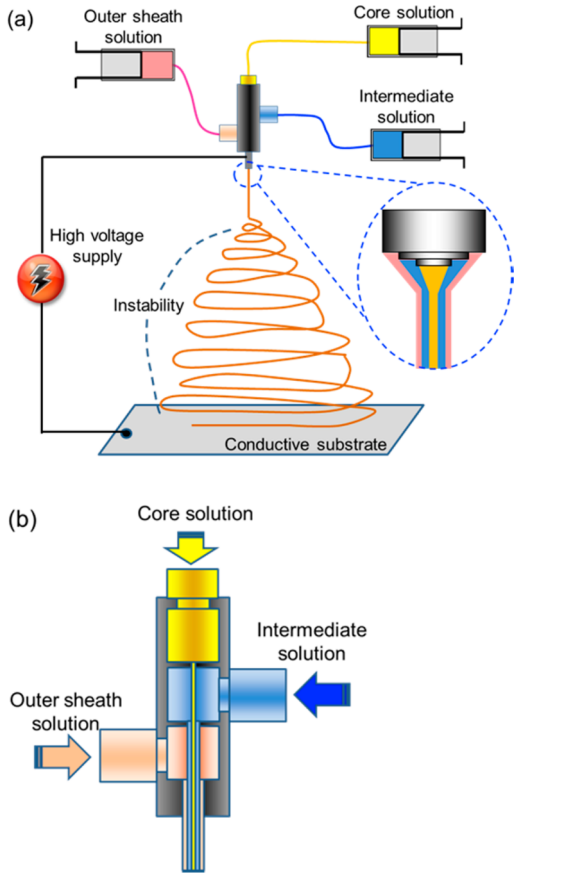

Figure 2. Triaxial electrospinning: (a) basic mechanism; (b) triaxial nozzle.

Materials. Poly(ϵ-caprolactone) (PCL, M_n = 80 kDa), sodium dodecyl sulfate (SDS), Triton X-102, phosphate buffered saline (PBS) salt were purchased from Sigma-Aldrich (St. Louis, MO). Polyvinylpyrrolidone (PVP) and 2,2,2,-trifluoroethanol (TFE, 99.8% purity) solvent were purchased from Acros Organics (Geel, Belgium) and chloroform solvent was purchased from TEDIA (Fairfield, OH). Salmon DNA (M_W = 100KDa) was generously provided by Biokom (Los Lagos, Chile). Keyacid uranine (KAU) (xanthene derivative) and Keyacid blue (KAB) (triphenylmethane derivative) dyes used as a model drug were purchased from Keystone (Chicago, IL). The absorption peaks of KAU and KAB dyes are at wavelengths of 628 and 488 nm, respectively. Because the two dyes have no overlap in absorption spectrum, it is very convenient to measure their absorption peaks for dual drug release experiments. All materials were used as received without any further modification.

Sample Preparation. The core solution was prepared as 15 wt % of PVP in deionized (DI) water and then added 0.6 wt % of KAB dye. The solution for the intermediate layer was prepared as 10 wt % of PCL in the mixture of chloroform (CF) and trifluoroethanol (TFE) in the ratio of 3:1. For the outermost (sheath) layer, the solution has been prepared by dissolving 10 wt % of PCL in TFE. For dual release experiment, 1 wt % of KAU dye was added to the PCL sheath solution and also used for single nozzle electrospinning for comparison purpose. Detailed composition and electrospinning parameters for all samples are shown in Table 1.

For sample C2, the reason for the change in solvent for PCL in the sheath was to demonstrate that the burst release from core resulted

Table 1. Electrospinning Parameters for All Samples Used. Dielectric Constants of the Solvents are 78 for H_2O, 26 for TFE, and 5 for CF

	triaxial fiber				coaxial fiber		single fiber
	T1	T2	T3	T4	C1	C2	S1
core/solvent	PVP+KAB/water	PVP+KAB/water	PVP+KAB/water	PVP+KAB/water	PVP+KAB/water	PVP+KAB/water	PCL+KAU/TFE
intermediate/solvent	PCL/CF:TFE (3:1)	PCL/CF:TFE (3:1)	PCL/CF:TFE (3:1)	PCL/CF:TFE (3:1)			
sheath/solvent	PCL+KAU/TFE	PCL+KAU/TFE	PCL+KAU/TFE	PCL+KAU/TFE	PCL/TFE	PCL+KAU/CF	
gap (cm)	25 cm	25 cm	25 cm	25 cm	25 cm	25 cm	25 cm
voltage (kV)	18−19	19	22	23	19−21	22	19−20
flow rate (mL/h)	core: 0.04 inter: 0.8 sheath: 0.2	core: 0.04 inter: 1.2 sheath: 0.2	core: 0.04 inter: 0.8 sheath: 0.2	core: 0.04 inter: 1.2 sheath: 0.2	core: 0.08 sheath: 0.4	core: 0.08 sheath: 0.4	0.4
dispensed volume (μL)	core: 30 inter: 600 sheath: 150	core: 30 inter: 900 sheath: 150	core: 30 inter: 600 sheath: 150	core: 30 inter: 900 sheath: 150	core: 30 sheath: 150	core: 30 sheath: 150	150

from the incorporation of water-soluble dye in the sheath layer rather than due to diffusion between the two solutions during coaxial electrospinning.

RESULTS

The formation of fiber mats using either coaxial or triaxial electrospinning of the materials indicated above proceeded with no experimental difficulties. To observe the fiber morphologies, SEM images were obtained using EVEX miniSEM at the acceleration voltage of 15KV. To prevent samples from charging, a very thin layer of gold was sputtered on samples for 1 min at the pressure of 50−60 mTorr using Desk II mini sputter machine (Denton Vacuum).

As shown in Figure 3, fiber morphologies are quite uniform, with no bead formation. Dual drug loaded triaxial fibers (Figure 3a) have a diameter of 648 nm with a standard deviation of 259 nm, while the other triaxial fibers (Figure 3b) have a slightly larger diameter of 702 nm with a standard deviation of 327 nm because of the increased flow rate of intermediate solution. Conventional "single" (i.e., noncoaxial) PCL fibers containing KAU dye molecules (Figure 3c) have a somewhat thinner diameter (360 nm ±83 nm) because of the absence of other layers. As expected, coaxial fiber with dual dyes (Figure 3d) has a fiber diameter of 582 nm ±161 nm, which is thinner than triaxial fibers (T1) but thicker than single fibers (S1).

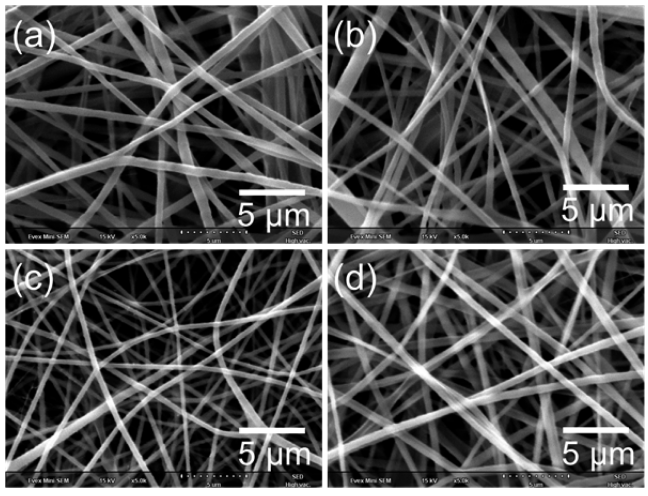

Figure 3. SEM microphotographs: dual dye-loaded (KAB in core and KAU in sheath) triaxial fibers produced with the intermediate flow rate of (a) 0.8 mL/h (T1) and (b) 1.2 mL/h (T2); (c) single PCL fibers with KAU dye (S1); (d) dual dye-loaded coaxial fibers (C2).

Using an FEI CM20 transmission electron microscopy (TEM) with acceleration voltage of 120KV, we have investigated the three layer structure of triaxially electrospun fibers. However, it is very hard to observe the triaxial strucutre on the triaxial fibers used for dual drug delivery because they contain the same material (PCL) for sheath and intermediate layers. Therefore, to observe the three layered structure we have used three different materials, DNA core, PCL intermediate layer and nylon6 outer sheath - for TEM observation and the triaxial structure was successfully observed, as shown in Figure 4.

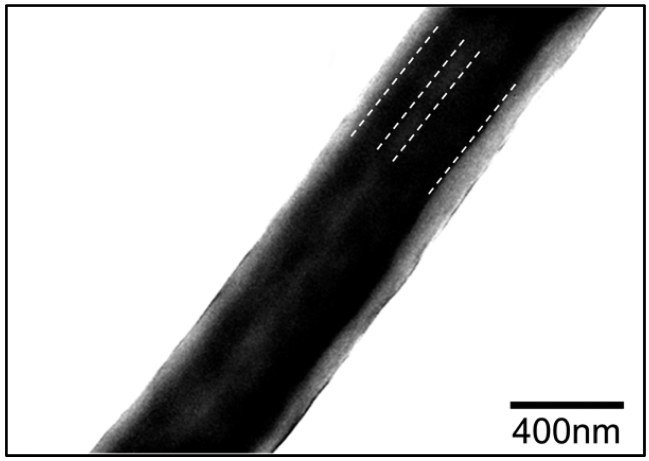

Figure 4. TEM observation for triaxial fibers made of DNA core, PCL intermediate layer, and nylon6 outer sheath.

The release behavior of fiber-encapsulated molecules was characterized using a Perkin-Elmer Lambda 900 UV/vis/NIR spectrometer. First, electrospun fiber mats were immersed into 50 mL of PBS solution (pH 7.4). Then the optical absorption spectrum of dye-containing solution sample was measured repeatedly for up to 50 h. The relative amounts of KAB and KAU dyes released from fiber samples were determined by measuring the height of absorption peaks of KAB and KAU dyes. Background subtraction was performed using samples of pure PBS solution.

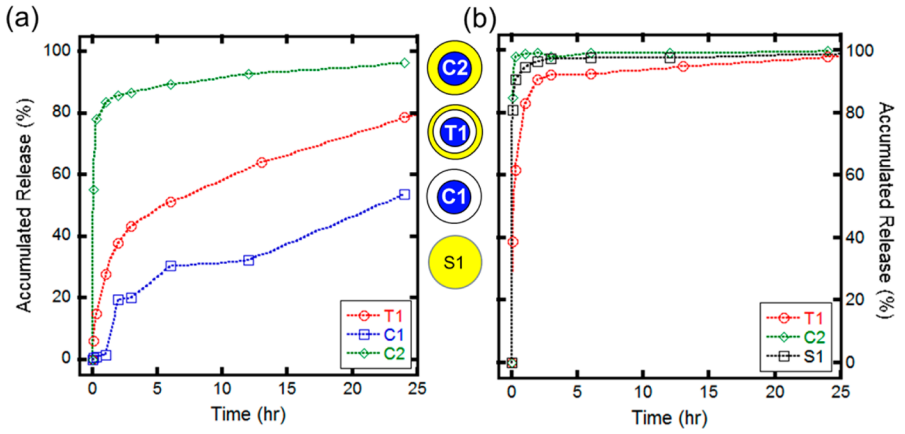

Figure 5. Comparison of drug release behaviors from different type of fiber mats: (a) KAB release from PVP core of coaxial/triaxial fiber mats; (b) KAU release from PCL sheath of coaxial/triaxial fiber mats or single fiber mat (S1). Inserts: Cross-sections of fibers are illustrated with indication of dye loading.

Results from experiments with the release of KAB from the fiber core and KAU dye from the sheath are compared in Figure 5. Triaxial fibers have been fabricated with both KAB dye in the PVP core and KAU dye in the PCL sheath. For comparison, a conventional "single" nozzle PCL fiber mat and coaxial fibers mats with or without KAU dye in sheath was fabricated, which incorporated the same amount of dyes.

As shown in Figure 5a, coaxial fiber loaded with dual dyes (C2) shows significant burst release of core dye with minimal sustained release. The coaxial fiber with one dye in core (C1) provides most sustained release from core. Incorporated KAU dye in outer sheath layer brought hygroscopic properties to the sheath. Hygroscopic sheath absorbs water and forms wet channels between core and outer environment, leading to burst release from core. To solve this problem, we have used triaxial electrospinning to produce triaxial fibers with dual dyes (T1), which show much improved sustained dye release and reduced burst release from core compared to the coaxial fibers with dual dyes (C2). For example, the coaxial fiber mat releases ~80% of the stored dye molecules in only 1 h whereas the triaxial fiber mat requires 24 h to reach the same dye release level. As expected, similar KAU dye release was obtained from the homogeneous single fiber (S1) and the outermost sheath layer of coaxial/triaxial fibers, with abrupt burst release and maximum saturation at an early stage, as shown in Figure 5b.

The dye release profile from the core of triaxial fiber mats can be controlled by modifying electrospinning parameters. Figure 6 shows the effect of core nozzle diameter and the flow rate of intermediate solution during electrospinning. Triaxial fiber mats were produced with narrower core nozzle diameter/wider intermediate gap (T1/T2) and wider core nozzle/thinner intermediate gap (T3/T4) shown schematically in Figure 6a. The flow rate used for the intermediate layer were 0.8 mL/h for T1 and T3 and 1.2 mL/h for T2 and T4. The combined effect of changing nozzle diameters and the intermediate flow rate results in good control of the core encapsulation and consequent dye release kinetics. As can be seen in Figure 6b, the release rate decreases with flow rate (T2 < T1, T4 < T3) and with the decrease in core nozzle inner diameter and increase in intermediate nozzle opening (T1 < T3, T2 < T4). As expected, KAU dye release from the sheath of triaxial fiber mats with different conditions shows similar abrupt release behavior (Figure 6c).

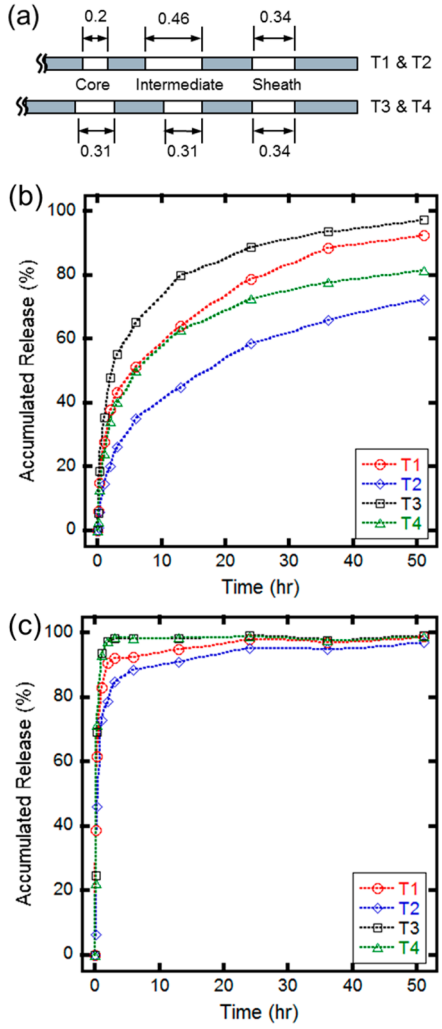

Figure 6. Effect of the core nozzle diameter and the flow rate of intermediate solution (0.8 mL/h for T1 & T3 samples; 1.2 mL/h for T2 & T4 samples): (a) schematic of triaxial nozzle dimensions; (b) KAB release from core of triaxial fiber mats; (c) KAU release from sheath of triaxial fiber mats.

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CONCLUSIONS

In this paper, we have successfully produced triaxial nanofiber membranes separately loaded with two model drugs in a single step. Our results represent the first trial to develop an advanced drug delivery system using multicoated fibers produced by triaxial electrospinning, Burst release of molecules in the sheath of triaxial fibers and controlled release from molecules encapsulated in the fiber core has been obtained due to the barrier effect of the intermediate layer. In future implementations, one can utilize fully hygroscopic biocompatible polymers and many selections of polar drugs in the sheath layer. Selecting a less water-miscible solvent for the intermediate material minimizes the diffusion of dye molecules between core and intermediate layers. The interactions between the various solvents and solutes used in multiaxial fiber electrospinning are listed in more detail in the Supporting Information (Scheme S1). Triaxial structured fibers have been shown to provide a degree of freedom in the selection of the polymers and drug molecules.

The triaxial electrospun nanofiber membranes loaded with two different types of functional molecules (such as drug, proteins and genes), represent a versatile method to realize ideal wound care products for both external and internal treatments by providing both short-term and long-term treatment from a single fibrous membrane. For example, wound dressings made of triaxial fibers, which have an outer sheath layer loaded with anesthetic drug and a core with antibiotic drug, can minimize pain at the early stage of the wound and prevent infection over the entire healing period. Another potential application is in cancer treatment, where two different drug and gene therapies can be combined by loading genes in the sheath and anticancer drugs in the fiber core.

Other potential alternatives for the outer layer include the use of stimuli-responsive polymers instead of the hygroscopic polymer so that the triggered (but sustained) release of drugs from core can be obtained based on pH/temperature changes in environment. Many other possible combinations can be established by incorporating nanoparticles or other organic molecules in the various layers of triaxial fibers.

ASSOCIATED CONTENT

Supporting Information

Combinations of solutes and solvents for successfully multiaxial electrospinning have been described. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: a.steckl@uc.edu.

Notes

The authors declare no competing financial interest.

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