

Encapsulation of Enzymes in Biodegradable Tubular Structures

Y. Dror,[†] J. Kuhn,[‡] R. Avrahami,[†] and E. Zussman^{*,†}

Faculty of Mechanical Engineering and Faculty of Biology, Technion, Israel Institute of Technology, Haifa 32000, Israel

Received July 18, 2007; Revised Manuscript Received March 7, 2008

ABSTRACT: Enzymes were directly encapsulated in microtubes fabricated by coelectrospinning. Coelectrospinning makes it possible to separate between an organic phase, which may be harmful to enzyme activity, and an aqueous one. In this report, enzymes were incorporated in an aqueous core solution of poly(ethylene oxide) (PEO) while the shell solution was made of polycaprolactone (PCL) dissolved in a mixture of chloroform and dimethylformamide (DMF). Indeed, this separation was found to preserve the enzymatic activities of two enzymes during and after electrospinning, as ascertained when the fiber mats were placed in an aqueous assay environment. The shell morphology was altered by blending PCL with poly(ethylene glycol) (PEG). This blending induced pores in the shell which in turn pronouncedly affected the movement of molecules into or out of the fibers. An enzyme of about 80 kDa, alkaline phosphatase (AP), diffused through the pores into the surrounding medium. The fibers can thus act as release devices. However, a larger enzyme, β -galactosidase (465 kDa), remained in the fibers without any leaching. Enzyme assays showed that the substrates of both enzymes diffused efficiently into the fibers and were cleaved by the encapsulated enzyme, and the reaction product then diffused outside. With encapsulated β -galactosidase, the fiber acted as an efficient enzymatic microreactor and the relative enzymatic activity reached a level of about 50%.

Introduction

Electrospun polymeric nanofibers have been widely used in biological applications such as scaffolds,^{1,2} carriers for biologically active molecules like proteins and enzymes,^{3–6} and host capsules for viruses and bacteria.⁷ The versatile use of such fibers is attributed to the size of the fibers which range from about 100 nm to several micrometers, the accompanying large surface area of such nanofibers, and the large variety of polymers from which they can be made. Recently, Sun et al.⁸ developed an electrospinning method that creates nanofibers with an outer shell and an inner core. Since the outer layer can be made of material insoluble in aqueous environments while the inner is soluble, a new range of possibilities has opened.^{9,10} The sequestration of enzymes by immobilization and embedment has a long and extensive history. The present research investigates the properties of core-shell nanofibers when enzymes are embedded in the inner layer.

The use of electrospinning for incorporation of enzymes has gained increasing attention. It is well-known that the immobilization of enzymes on solid substrates offers advantages over the use of free enzyme. Immobilization can stabilize enzymes, enable better control of an enzymatic reaction, and allow their repeated use. The host material has a significant role in evoking these advantages from both the architectural and chemical points of view. Electrospun nanofibers are excellent candidates for hosting enzymes for several reasons: (1) their small size confers a large surface area and makes possible the creation of materials with high concentrations of an enzyme, (2) the porosity of the fibers can be tailored, and thus the movement of molecules into and out of the fibers can be controlled according to specific requirements, (3) the use of nonsoluble fibrous mats in aqueous medium provides the ability to recover the mat for reuse, and (4) multiple enzymes from diverse sources can be encapsulated together. So far, two main methods have been employed to make an assemblage of enzymes and polymeric fibers via electrospinning. The first of

these is the immobilization of the enzyme on the outer surface of the nanofibers. Immobilization is carried out by initially spinning the polymer solution and then either covalently attaching the desired enzyme(s) to the polymer surface which possesses functional groups by introducing the fibers to an enzyme solution^{11–13} or by the physical adsorption of the enzyme to the surface as has been shown by Huang and co-workers.¹⁴ The main advantage of these procedures is the actual attachment of the enzyme which prevents its loss with time. However, the enzyme is anchored and active only on the outer surface of the fibers while the interior of the fibers remains inactive. Moreover, the exposure of the enzyme to its surroundings may have ruinous effects on enzyme activity. In an alternate approach, the enzyme is mixed with the polymer solution and subsequently spun. In contrast to the first approach, we refer to this process as encapsulation rather than immobilization. In the case of the widely used water-soluble polymers such as poly(vinyl alcohol) (PVA) or dextran for biological applications, the mat can be cross-linked by chemical or physical agents such as glutaraldehyde^{15,16} or UV irradiation¹⁷ to prevent the immediate dissolution of the fibers in an aqueous environment and subsequent enzyme leaching. A different solution to the fibers dissolution problem was suggested by Zeng and co-workers who coated the PVA fibers with a water-insoluble polymer by chemical vapor deposition (CVD).¹⁸ The main weaknesses of the direct encapsulation method are obviously the leaching of the enzyme which is pronounced when the host polymer is water soluble and the exposure of the enzyme to harmful organic solvents when water-insoluble polymers are used. To overcome this problem, it was suggested by Herricks et al. to use surfactant-stabilized enzymes in an organic solution of polystyrene (PS) as a spinning solution. In this way, the electrospun nanofibers are insoluble in water and the enzymatic activity is retained due to surfactant stabilization.¹⁹

In this work, we present a third and novel approach whereby enzymes are encapsulated within electrospun fibers using a core-shell method. This approach overcomes both the problem of fiber dissolution and subsequent leaching of the enzyme on the one hand and the exposure of the enzyme to harmful solvents on the other. The enzyme is incorporated in an aqueous polymeric

* Corresponding author. E-mail: meeyal@technion.ac.il.

[†] Faculty of Mechanical Engineering.

[‡] Faculty of Biology.

Table 1. Two Types of Core–Shell Fibers: Composition of the Solutions

type	shell solution	core solution
1	10% (w/w) PCL 80K CHCl ₃ :DMF 90:10	4% (w/w) PEO 600K + 73 μ g/mL AP or 2.38 units/mL β -GAL in ethanol:H ₂ O 26:74
2	10% (w/w) PCL 80K +1% PEG 6K CHCl ₃ :DMF 90:10	4% (w/w) PEO 600K + 73 μ g/mL AP or 2.38 units/mL β -GAL in ethanol:H ₂ O 26:74

Table 2. Composition of the Assays

enzyme	substrate	buffer	H ₂ O	products
AP	0.7 mg/mL <i>p</i> -nitrophenylphosphate (MW = 217) 1 mL 4-methylumbelliferylphosphate (for fluorescence microscopy)	TRIS-HCl buffer 1.5 mL pH 8.3	0.5 mL	<i>p</i> -nitrophenol + PO ₄ ³⁻ 4-methylumbelliferone + PO ₄ ³⁻
β -GAL	4 mg/mL <i>o</i> -nitrophenyl- β -D-galactoside (MW = 301) 0.2 mL 4-methylumbelliferyl- β -D-galactoside (for fluorescence microscopy)	Z-buffer 0.7 mL (ref 24)	0.3 mL	<i>o</i> -nitrophenol + galactose 4-methylumbelliferone + galactose

core solution while the shell solution is composed of a water-insoluble polymer which forms an external protective layer. In particular, we have used polycaprolactone (PCL) dissolved in a mixture of dimethylformamide (DMF) and chloroform as the shell solution and poly(ethylene oxide) (PEO) dissolved in a mixture of water and ethanol as the core solution. Alkaline phosphatase (AP) and β -galactosidase (β -GAL), two water-soluble enzymes with different molecular weights, have been investigated. This pair of polymers results in the formation of hollow fibers, i.e., microtubes.²⁰ In the present paper, we describe the use of these microtubes as enzymatic microreactors where the inner space enables a confined space without restricting the freedom of the enzyme to assume different conformations or movement via diffusion. The substrate diffuses through the shell to the inner space where the enzymatic reaction takes place and the product can then diffuse out. In addition, the influence of the morphology of the shell on reaction kinetics is presented. The morphology of the shell was modified by blending the shell solution with poly(ethylene glycol) (PEG).

Experimental Section

Materials and Solutions. The compositions of the shell and core solutions are given in Table 1. All polymers and solvents were purchased from Sigma-Aldrich and were used as is. AP and β -GAL from *E. coli* were also purchased from Sigma-Aldrich. AP is a homodimer and cleaves monophosphate esters and has a molecular weight of about 80 kDa. β -GAL is a tetrameric enzyme of 465 kDa consisting of four identical subunits each²¹ and catalyzes the hydrolysis of the terminal galactosidyl group of β -galactosides. Both enzymes were initially dissolved in water and then mixed with the core solution.

Electrospinning. Core–shell fibers were fabricated by a coelectrospinning process using the setup described by Sun et al.⁸ and Zussman et al.²² All experiments were conducted at room temperature (~22 °C) and a relative humidity of about 35%. The spinning parameters were as follows: the electrostatic field used was approximately 0.44 kV/cm, and the distance between the spinneret and collector plate was 16 cm. The flow rates of both the core and shell solutions were controlled by two syringe pumps and were 3.5 mL/h for the shell and 1 mL/h for the core. The fibers were collected as a strip on the edge of a vertical rotating wheel²³ having a velocity of 1.2 m/s. For fluorescence microscopy, a few fibers were collected directly onto a microscope slide.

Imaging. Images of the fibers were obtained using a Leo Gemini high-resolution scanning electron microscope (HRSEM) at an acceleration voltage of 3 kV and a sample to detector distance of 3–5 mm. The specimens were coated with a thin gold film to increase their conductivity.

Fluorescence microscope Leica DM IRE2 at excitation and emission wavelengths of 359 and 361 nm, respectively, was used for the imaging of fibers filled with a fluorescent product of an enzymatic reaction.

Measurement of Enzyme Activity. To measure enzyme activity, pieces of mat were weighed and dipped in an assay solution described in Table 2. At each time of sampling, the solution was mixed with a Vortex mixer, and 1 mL of the assay mixture was

transferred to spectrophotometer cuvette. The absorbance of the solution was measured in a Perkins-Elmer spectrophotometer at a wavelength of 410 nm. For both enzymatic reactions, the substrates are colorless but the product, nitrophenol, is yellow with an absorption maximum at 410 nm. After the absorbance was measured, the liquid was returned to the assay vessel. Units, activity, and relative activity are defined as follows:

$$\text{units} = \frac{\Delta A}{\Delta t} \times 1000 \quad (1)$$

$$\text{activity of the mat} = \text{unit} \times C \quad (2)$$

where

$$C = \frac{\text{mass of total mat}}{\text{mass of piece}} \quad (2a)$$

$$\text{relative activity (\%)} = \frac{\text{activity of the mat}}{\text{activity of the core solution}} \times 100 \quad (3)$$

ΔA is the difference in the absorbance between different time points, Δt is a time interval in the linear region of the reaction curve, and C is a normalization factor which takes into account the different weight of each piece of mat assayed.

For the fluorescence microscope imaging, a drop of the assay solution was put directly on the microscope slide on which a few fibers had been deposited. The fluorescent substrates were methylumbelliferyl phosphate for AP and methylumbelliferyl galactoside for β -GAL. These were used at the same concentrations as their nitrophenyl analogues.

Results and Discussion

Two types of core–shell fibers have been fabricated with the polymers listed in Table 1. The resultant fibers are hollow structures, namely microtubes, as has been thoroughly discussed in a previous publication.²⁰ The hollow nature of these structures and the different morphologies of the tube walls are clearly shown in the SEM micrographs presented in Figure 1. Type 1 fibers are made with only PCL in the shell and exhibit a rough surface due to the rapid evaporation of the solvents, chloroform, and DMF which is a common phenomenon²⁵ when using volatile organic solvents (Figure 1, a and c). However, this roughness does not affect the intact nature of the fiber walls. As PEG is added to the shell solution (type 2), the walls become increasingly porous and pores are seen even on the interior surface of the tubes (Figure 1, b and d), as previously described by Jiang et al.⁵ PEG and PCL are somewhat miscible due to favorable, but weak, intermolecular polar interactions.²⁶ During fiber solidification, i.e., the evaporation of the solvents, the concentration of these two components increases and phase separation takes place. However, since PEG has a surfactant-like character, it deposits an adherent film around the PCL domains and this results in pore formation rather than the formation of solid domains of PEG.

The kinetics of the alkaline phosphatase reaction were measured as described in the Experimental Section and are presented in Figure 2. The enzymatic activity of the fibers was

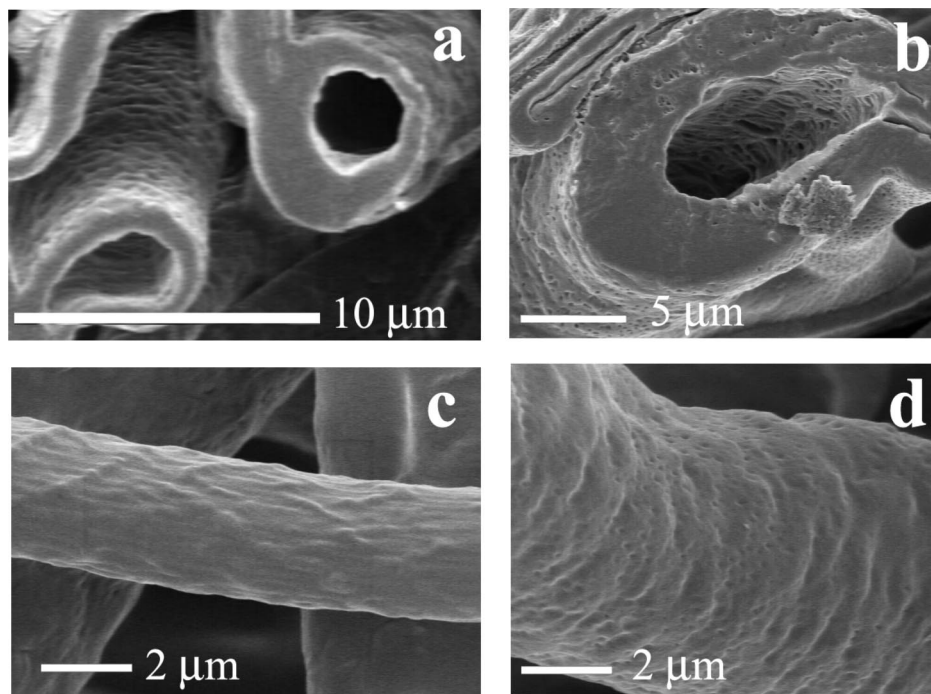


Figure 1. HRSEM micrographs of (a) type 1 fibers with AP, (b) type 2 fibers with AP, (c) type 1 fibers with β -GAL, and (d) type 2 fibers with β -GAL.

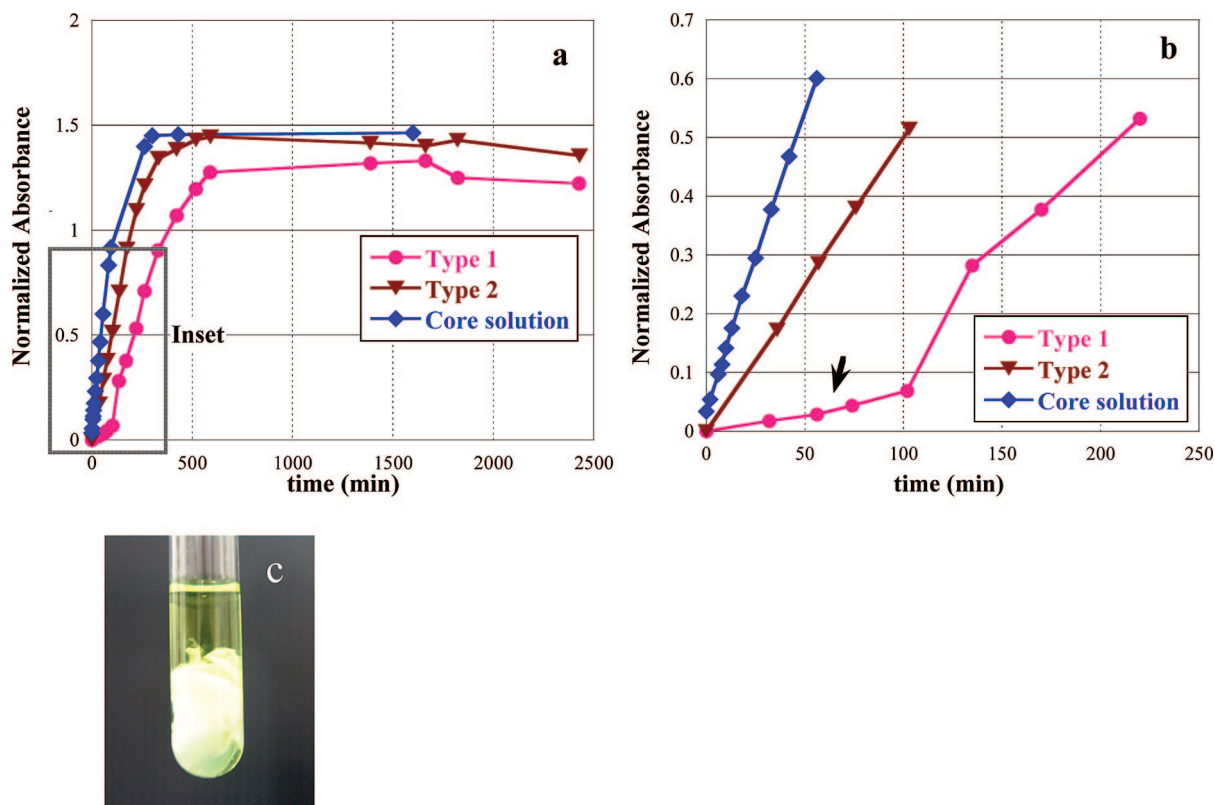


Figure 2. (a) Progress of the AP reactions with time for the electrospun fibers and the free enzyme in the core solution, (b) inset, (c) photograph of a piece of mat immersed in the assay solution. The presence of the yellow product, *p*-nitrophenol, is apparent.

compared to that of the enzyme in the core solution (before electrospinning) and then normalized with regard to the weight of the analyzed pieces. The results clearly show that encapsulated enzymes in both types of fiber maintain their biological activity after spinning and exhibit reaction rates similar to the free enzyme. The curves are characteristic of enzymatic reactions in the presence of a large excess of substrate. The colored

product, *p*-nitrophenol, diffuses out of the fibers into the surrounding medium as shown in Figure 2c. The reaction rates of the encapsulated enzyme are reduced in comparison to that of the free enzyme. Their rates may be reduced due to one or more of the following reasons: (1) the substrate has to diffuse into the fibers in order to reach the active site of the enzyme; and/or (2) the enzyme has to diffuse outside to become active;

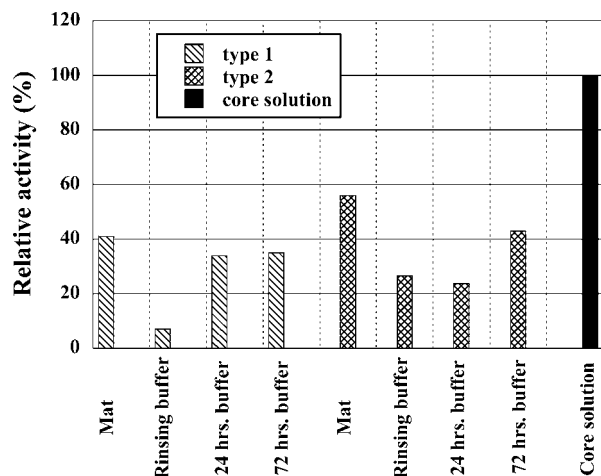


Figure 3. Relative activity of AP enzyme for different types of fibers and in dwelling buffers.

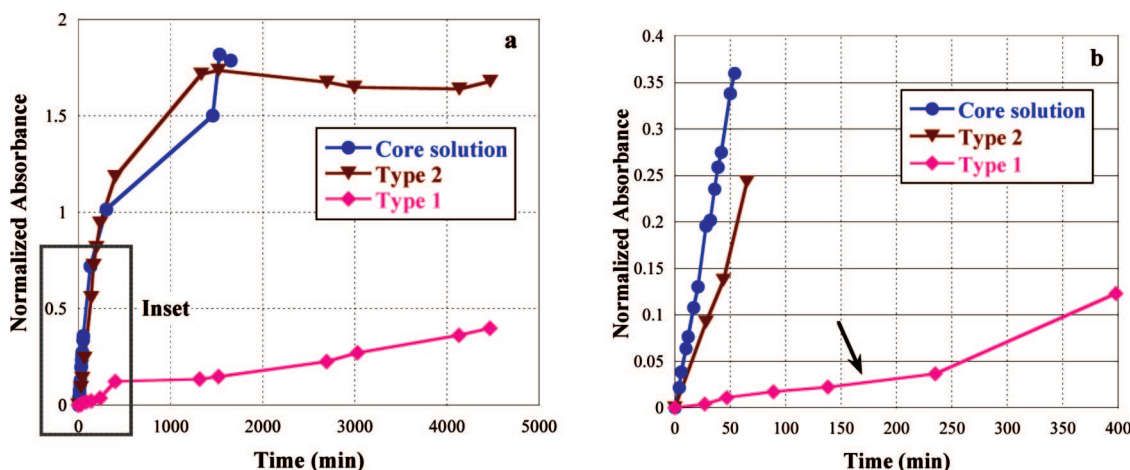


Figure 4. (a) Progress of the β -GAL reactions with time for the two types of electrospun fibers and the free enzyme in the core solution; (b) inset.

(3) the product has to diffuse outside in order to be detected; (4) some enzymatic activity was lost during the spinning process itself. The attainment of the maximum reaction velocity is seen to occur immediately with type 2 fibers which undoubtedly stems from their highly porous character. Upon closer examination of the initial kinetics, it can be seen that type 1 fibers, which do not contain PEG in the shell, exhibit a linear reaction rate that is initially much reduced (inset in Figure 2b, marked by a dark arrow). In the porous fibers (type 2) the penetration of the substrate is unencumbered. The presence of PEG probably facilitates the wetting of the outer surface of the fibers and thereby allows the access of this hydrophilic substrate.

In order to determine whether the enzyme diffuses out of the fibers, three specimens containing AP were cut out of both types of nonwoven mats and placed in the buffer. The buffer without the fibers from the first sample was taken immediately and assayed for enzymatic activity (rinsing). For the second and third samples, the buffer was analyzed after 24 and 72 h, respectively. The first sample was tested in order to evaluate if any removable enzyme resides on the outer surface of the fibers after the spinning. The relative activity of the buffers for the two types is presented in Figure 3. Initially, some alkaline phosphatase is present in the buffer and this signifies that some of the enzyme resides either on the outside of the fibers or is readily lost because it is in or near the pores. A significant fraction of the enzyme has leached out of the fibers within the first 24 h. For type 1 fibers about 80% of the enzyme has eluted at this time and there is little further loss during the next 48 h. Type 2 fibers

exhibit a relatively high level of enzyme that is released immediately and this increases to about 74% at 72 h. Therefore, it is clear that some enzyme is located on the outer surface of the fibers and can immediately enter the surrounding buffer upon rinsing as was previously demonstrated with regular nanofibers by Srikanth et al.²⁷ The migration of the enzyme to the outer surface of the fibers during the spinning is more pronounced in type 2, due almost certainly to the presence of PEG which might serve as hydrophilic conduit for the enzyme. It has already been shown²⁸ that all charges immediately accumulate at the outer surface of the shell in the drop in the core-shell electrospinning process. Charged ions preferentially migrate as the electric field is applied. While there is no overall charge on proteins at their isoelectric point, they do have an overall charge at pHs below and above this point. Proteins that have an overall positive charge in the core solution will migrate toward the free surface of the drop since the cathode is immersed in the core solution. This may explain the relatively large quantity of alkaline phosphatase that was released by rinsing the mats.

Utilizing the same methodology, the kinetics of the β -GAL reaction were determined in the two types of electrospun mats and the results of these analyses are presented in Figure 4. The figure shows that type 2 fibers have almost as much activity as that put into them initially. Type 1 fibers, in contrast, exhibit a relatively low amount of enzymatic activity that remains fairly constant throughout the experiment.

The release of β -GAL from the fibers was examined to answer some of the questions posed above. Pieces of mat were

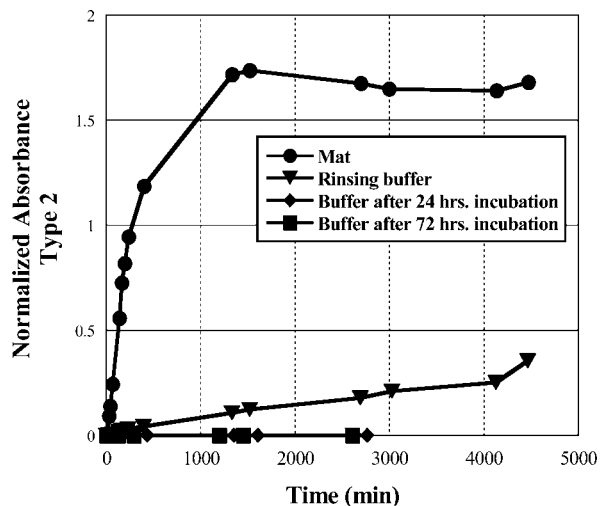


Figure 5. β -GAL reaction versus time for the mat and buffers for type 2 fibers.

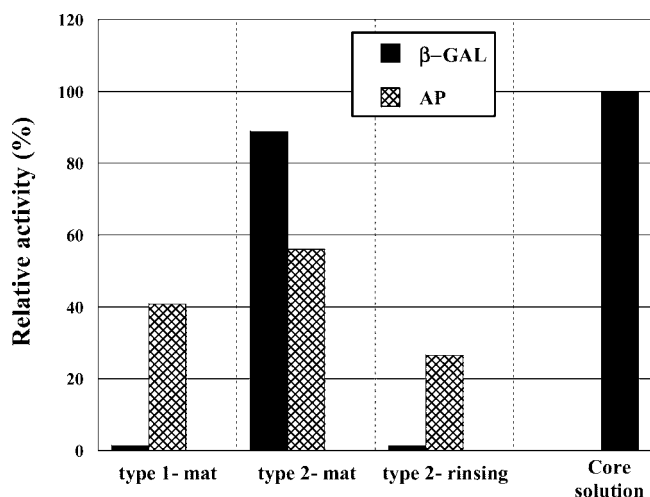


Figure 6. Relative activity of the β -GAL and AP for different types of fibers.

immersed in buffer and assayed for enzymatic activity at 0, 24, and 72 h to determine whether there had been any leaching of the enzyme. Figure 5 demonstrates that there is only a negligible loss of the enzyme from the fibers even for those of type 2 which should show a greater loss of enzyme as discussed above for AP. In both types of fibers, β -GAL remains within the fibers. This allows one to draw inferences about substrate and product diffusion into and out of these fibers. Since it seems unlikely that the great difference between the types with respect to activity stems from an effect of PEG on protecting the enzyme, this difference almost certainly reflects the ease of diffusion of the substrate and/or the product. In type 1 fibers, small hydrophilic molecules have difficulty penetrating to the lumen while PEG and the pores created by its presence greatly increase the rate of diffusion in type 2 fibers. β -GAL remains fiber-associated throughout the 72 h immersion of a mat in buffer, reflecting its much greater molecular weight. The lack of an initial loss of the enzyme immediately after spinning seems to be due to its charge under the specific conditions employed.

A comparison of the two enzymes with respect to fiber type and their behavior during spinning is presented in Figure 6. It is clear that for type 2 fibers with β -GAL, the reaction takes place only within the fibers. The electrospinning of β -GAL creates hollow fibers that act as a microreactor in which the substrate enters the "reactor" through the porous shell and is

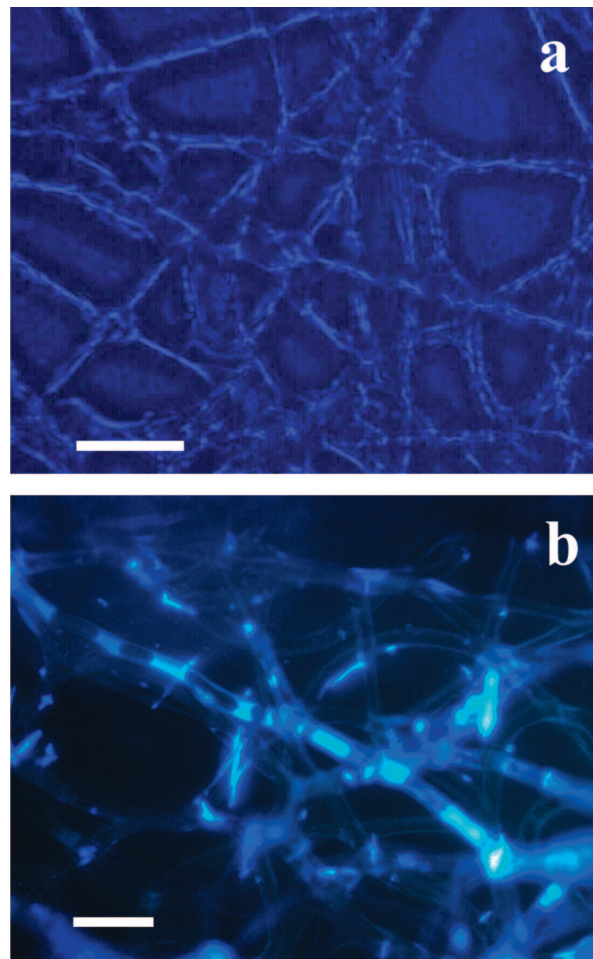


Figure 7. Fluorescence microscope micrographs of type 1 fibers with (a) AP, bar = 100 μ m, and (b) β -GAL, bar = 50 μ m.

cleaved by the encapsulated enzyme, and the reaction product then diffuses out into the surrounding medium. Thus, the reduction in the reaction rate for type 1 β -GAL fibers seems to be related directly to the slow diffusion of the substrate into these fibers. The size of an enzyme apparently affects the amount of its subsequent discharge into the surrounding medium. With β -GAL fibers, only a small amount of enzyme was detected in the rinsing buffer for type 2 while for type 1 no enzyme leached out (not shown). Type 1 fibers exhibit reduced reaction rates for both enzymes and especially for β -GAL. Type 1 fibers are hydrophobic and less porous than those of type 2, and they inhibit the entrance of the substrates used here. It would seem that type 1 fibers will be less efficacious as flow-through reactors, in contrast to the remarkably efficient systems obtained with type 2 fibers.

Visual evidence that the enzymatic reaction occurs within the fibers was obtained by using substrates in which one of the products is fluorescent. For both enzymes the substrates used liberate 4-methyumbelliferone after hydrolysis which allows imaging by fluorescence microscopy. As is clearly seen in Figure 7 when the fibers are immersed in these substrates, the interior of the fibers is fluorescent while the surrounding medium is dark. This shows that both enzymatic reactions, in fact, take place within the type 1 fibers. However, it is important to emphasize that, in contrast to the mat immersion experiments, this method is very sensitive and enables the detection of very small amounts of product which can occur within a relatively short time. Indeed, these images were acquired within 1–2 min after the substrate was applied, a time scale which is greater than the characteristic time of the diffusion through the

microtube wall—in the order of 10 s as calculated in our previous report.²⁰ Hence, these results are not in contradiction to those of the mat immersion experiments in which the kinetics were followed over a much longer period during which both the product and the enzyme (in the AP case) can diffuse outside the fibers. In Figure 7b, short slugs (sections) of fluorescent liquid are observed. This phenomenon has been previously found in such fibers.²⁰

Conclusion

Enzymes were directly incorporated in microtubes fabricated by coelectrospinning by introducing the enzymes in an aqueous core solution of PEO. The shell solution was made of PCL dissolved in a mixture of chloroform and DMF. The separation between the outer organic and inner aqueous phases was found to preserve enzyme activity during and after spinning when the mats were placed in an aqueous environment.

Two types of microtubes were fabricated which differ in their shell morphology. Type 2 shells were produced by adding PEG to the shell solution while type 1 fibers were made without this addition. By using a mixture of PEG and PCL to form the shell, pores were formed during the solidification process and this in turn directly affected the transfer of small molecules into and out of the fibers. As a consequence, the more porous fibers (type 2) exhibited higher rates of enzymatic reaction. In addition, two enzymes differing in their molecular weight were incorporated: AP and β -GAL. The difference in the molecular weight between the enzymes was well reflected in the kinetics of their release from the fibers of both kinds of microtubes. AP can diffuse out of the fibers while β -GAL remains fiber associated and its activity reflects the rate of substrate entry into the fibers. In other words, the AP fibers act as an enzyme release device in which the release rate can be tailored by modifying the morphology of the shell. The β -GAL fibers, on the other hand, act as an enzymatic microreactor and type 2 fibers exhibit a high level of enzymatic activity which shows that substrate penetration is extremely good with this kind of fibers. Once again, substrate availability and the release of the reaction products should be able to be controlled by manipulating the morphology of the shell. This method of encapsulation should find use when a separation between an enzyme or enzymes and the external aqueous environment is desired (e.g., with living tissue to avoid immunological reactions). The remarkable retention and enzymatic activity of β -GAL type 2 fibers clearly demonstrates that coelectrospinning can sequester an enzyme while preserving its activity.

Another advantage of these core-shell fibers is the small volume within the microtubes which enables the rapid buildup of the concentration of a product within them. This is extremely important for enzymes carrying out sequential reactions where the local concentration of the product of the first reaction serves as the substrate for a subsequent reaction. In this regard, these nanotubes are somewhat analogous to living cells except that any manner of enzymes may be added to the fibers without regard to their biological origin. This should lead to far more efficient processes than can be achieved by enzyme immobilization and in open vessels and this idea is presently under investigation. Another advantage of this system is that, unlike living cells, there is no discrimination as to which type of small

molecules may enter these tubes. For example, phosphorylated molecules (like *p*-nitrophenyl phosphate) do not enter *E. coli* cells.

Since these mats are insoluble in aqueous solutions, they should provide an excellent tool for the construction of biosensors and perhaps find use in enzyme therapy. We have also succeeded in encapsulating bacterial cells in such fibers which should open a new avenue to a range of products such as water purification, concentrating minerals, and flow-through biochemical syntheses and these results will be presented in another report.

References and Notes

- (1) Luu, Y. K.; Kim, K.; Hsiao, B. S.; Chu, B.; Hadjiargyrou, M. *J. Controlled Release* **2003**, *89*, 341–353.
- (2) Shin, H. J.; Lee, C. H.; Cho, I. H.; Kim, Y. J.; Lee, Y. J.; Kim, I. A.; Park, K. D.; Yui, N.; Shin, J. W. *J. Biomater. Sci., Polym. Ed.* **2006**, *17*, 103–119.
- (3) Xie, J.; Hsieh, Y. L. *J. Mater. Sci.* **2003**, *38*, 2125–2133.
- (4) Zhang, Y. Z.; Wang, X.; Lim, C. T.; Ramakrishna, S. *Biomacromolecules* **2006**, *7*, 1049–1057.
- (5) Jiang, H.; Hu, Y.; Zhao, P.; Li, Y.; Zhu, K. *J. Biomed. Mater. Res., Part B: Appl. Biomater.* **2006**, *79B*, 50–57.
- (6) Patel, A. C.; Li, S.; Yuan, J. M.; Wei, Y. *Nano Lett.* **2006**, *6*, 1042–1046.
- (7) Salalha, W.; Kuhn, J.; Dror, Y.; Zussman, E. *Nanotechnology* **2006**, *17*, 4675–4681.
- (8) Sun, Z. C.; Zussman, E.; Yarin, A. L.; Wendorff, J. H.; Greiner, A. *Adv. Mater.* **2003**, *15*, 1929–1936.
- (9) Greiner, A.; Wendorff, J. H.; Yarin, A. L.; Zussman, E. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 387–393.
- (10) Yarin, A. L.; Zussman, E.; Wendorff, J. H.; Greiner, A. *J. Mater. Chem.* **2007**, *17*, 2585–2599.
- (11) Ye, P.; Xu, Z. K.; Wu, J.; Innocent, C.; Seta, P. *Macromolecules* **2006**, *39*, 1041–1045.
- (12) Jia, H.; Zhu, G.; Vugrinovich, B.; Kataphinan, W.; Reneker, D. H.; Wang, P. *Biotechnol. Prog.* **2002**, *18*, 1027–1032.
- (13) Kim, T. G.; Park, T. G. *Biotechnol. Prog.* **2006**, *22*, 1108–1113.
- (14) Huang, X. J.; Xu, Z. K.; Wan, L. S.; Innocent, C.; Seta, P. *Macromol. Rapid Commun.* **2006**, *27*, 1341–1345.
- (15) Ren, G.; Xu, X.; Liu, Q.; Cheng, J.; Yuan, X.; Wu, L.; Wan, Y. *React. Funct. Polym.* **2006**, *66*, 1559–1564.
- (16) Wu, L.; Yuan, X.; Sheng, J. *J. Membr. Sci.* **2005**, *250*, 167–173.
- (17) Jiang, H.; Fang, D.; Hsiao, B. S.; Chu, B.; Chen, W. *Biomacromolecules* **2004**, *4*, 326–333.
- (18) Zeng, J.; Aigner, A.; Czubyko, F.; Kissel, T.; Wendorff, J. H.; Greiner, A. *Biomacromolecules* **2005**, *6*, 1484–1488.
- (19) Herricks, T. E.; Kim, S. H.; Kim, J.; Li, D.; Kwak, J. H.; Grate, J. W.; Xia, Y. N. *J. Mater. Chem.* **2005**, *15*, 3241–3245.
- (20) Dror, Y.; Salalha, W.; Avrahami, R.; Zussman, E.; Yarin, A. L.; Greiner, A.; Wendorff, J. H. *Small* **2006**, *3*, 1064–1073.
- (21) Zabin, I.; Flower, A. V. *β -Galactosidase, the Lactose Permease Protein and Thiogalactoside Transacetylase*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1980.
- (22) Zussman, E.; Yarin, A. L.; Bazilevsky, A. V.; Avrahami, R.; Feldman, M. *Adv. Mater.* **2006**, *18*, 348–353.
- (23) Theron, A.; Zussman, E.; Yarin, A. L. *Nanotechnology* **2001**, *12*, 384–390.
- (24) Miller, J. H. *Experiments in Molecular Genetics*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1972.
- (25) Casper, C. L.; Stephens, J. S.; Tassi, N. G.; Chase, D. B.; Rabolt, J. F. *Macromolecules* **2004**, *37*, 573–578.
- (26) Coleman, M. M.; Graf, J. F.; Painter, P. C. *Specific Interactions and the Miscibility of Polymer Blends*; Technomic Publishing Co.: Lancaster, PA, 1991.
- (27) Srikar, R.; Yarin, A. L.; Megaridis, C. M.; Bazilevsky, A. V.; Kelley, E. *Langmuir* **2008**, *24*, 965–974.
- (28) Reznik, S. N.; Yarin, A. L.; Zussman, E.; Berkovici, L. *Phys. Fluids* **2006**, *18*, 062101.

MA071599R