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Silica/PVA biocatalytic nanofibers

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ABSTRACT

Bioencapsulation has been a promising technique for various biotechnological and medical applications. Nevertheless, when encapsulated, the activity of the encapsulated biologicals is usually reduced due to diffusional resistance and use of organic solvents in the process. Here, we developed a sol–gel electrospinning technique to encapsulate a bacterium, *Escherichia coli* expressing a biocatalyst, AtzA, into hybrid silica/polyvinyl alcohol nanofibers. We used a microfluidic timer to maintain a constant sol viscosity, thus enabling continuous formation of silica gel nanofibers weaving a bioreactive mat. Encapsulation of bacteria into thin-walled (a few tens of nanometers) fibers significantly reduced diffusional resistance. Furthermore, with this process, the need for organic solvents was eliminated. This enabled us to reach encapsulated bacteria activity at the levels seen in free cells. This novel process enables new large-scale applications in biotechnology, especially in bioremediation, biosensors, and manufacturing of recombinant proteins.

1. Introduction

Bioencapsulation, which involves encapsulation of biologically active substances or cells in a permeable medium, has received considerable attention over the past decade. To date, various substances, including DNA, proteins, viruses, live bacteria and mammalian cells have been encapsulated for a wide spectrum of applications in medicine, biosensing, bioremediation, agriculture, biocatalysis, *de novo* synthesis of metabolites, and even synthesis of recombinant proteins [1]. In the field of bioremediation, although bacteria can be used to clean up environmental contaminants on the field, they cannot be localized to a specific region of interest when floating freely in solution and it is hard to maintain their activity against various environmental challenges such as temperature and pH. Therefore, encapsulation of bacteria is an appealing technology for the removal of herbicides, heavy metals, unmetabolized drugs, hydrocarbons and radioactive wastes. Diverse encapsulating materials and microbes have been used for bioremediation [2]. However, one common shortcoming of the previous efforts is the very low reaction rates obtained mainly due to diffusion limitations for the chemicals and metabolites.

Electrospinning is a promising approach to minimize diffusion limitations because bacteria can be encapsulated into thin-walled nanofibers [3]. However, electrospinning typically requires use of organic solvents and may hamper the catalytic activity. Several research groups, aiming to encapsulate reactive bacteria into electrospun nanofibers, investigated the use of organic solvents with low toxicity [4] or coaxial electrospinning to minimize the contact of organic solvent molecules with bacteria [5].

Silica-based materials have been widely used for bioencapsulation because of their thermal stability, relative inertness, low cost and biocompatible sol–gel transition at room temperature [6]. Different silicon alkoxides, including tetraethyl orthosilicate (TEOS) [7], tetramethyl orthosilicate (TMOS) [8], and a TEOS/aminopropyl triethoxysilane (APTES) mixture [9], have been used for sol–gel electrospinning. However, a major limitation of using TEOS or TEOS/APTES as silica precursor to make nanofibers is the essential use of an organic solvent (ethanol). It was reported that using TMOS as silica precursor, an organic solvent was not required for sol–gel electrospinning [10]. However, continuously changing viscosity of the solution caused limited scalability of the system. Due to these limitations, as to date, encapsulation of bacteria into electrospun silica or silica-based nanofibers was not reported.

In the current study, we developed a solvent-free sol–gel electrospinning technique coupled with a microfluidic timer. We also demonstrate the ability of the bacterium-encapsulated nanofibers to retain their morphology and enzymatic activity in an aqueous medium.

2. Experimental

TMOS, hydrochloric acid (HCl), and polyvinyl alcohol (PVA) were purchased from Sigma-Aldrich. PVA was dissolved in water. TMOS was hydrolyzed by sonication in the presence of HCl and the molar ratio of TMOS/water/HCl was 1:2.8:0.00024. *E. coli* expressing GFP were encapsulated for the fluorescence microscopy study

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and *E. coli* expressing atrazine chlorohydrolase (AtzA) were encapsulated for atrazine bioremediation study.

Table 1 summarizes the parameters investigated. For bioencapsulation, the PVA solution was mixed with *E. coli* pellets at a cell density of 0.5 g/ml. Fig. 1a shows the schematic diagram of the microfluidic timer, which was composed of a Y-shaped adapter, a capillary and a needle. The bacteria were dispersed in the PVA solution that reacted with the silica precursor in the microfluidic timer. As the fluid flowed through the capillary, its viscosity increased because of the increased reaction duration. The length of the capillary was adjusted such that the fluid reached the optimum viscosity for electrospinning at the orifice of the needle. At the orifice, the bacteria were pulled into a jet together with the polymer chains by an electrostatic force. As the optimum viscosity of the fluid was maintained at the orifice of the needle throughout the process, large-scale production of nanofibers was allowed. After evaporation of solvent, the bacteria were found to be encapsulated by a thin layer of silica/PVA (Fig. 1b).

Silica/PVA nanofibers fabricated using different combinations of parameters were examined by SEM (Hitachi S-4700). Each SEM micrograph was analyzed using an image analysis program (ImageJ) and the average fiber diameter was determined. The viscosity of the corresponding solution was measured using a viscometer (NDJ-8S). The GFP expression levels of *E. coli* before and after encapsulation were analyzed by fluorescence microscopy (Olympus BX50). The silica/PVA hybrid nanofibers with encapsulated AtzA *E. coli* bacteria were also examined by SEM and the average fiber diameter was measured. The free bacteria and encapsulated bacteria were examined by TEM (FEI Tecnai G^2 F30). Fourier transform infrared spectroscopy (FTIR) analysis was conducted for silica/PVA nanofibers with no bacteria, silica/PVA nanofibers with encapsulated *E. coli* bacteria, and silica/PVA with known percentages of *E. coli* bacteria, using a Nicolet Continuμm FTIR microscope. Activity measurements of the encapsulated bacteria were conducted by exposing the bacterium-encapsulated nanofibers to 5 ml of 0.1 M potassium phosphate buffer (at pH 7.0) containing 150 μM (32.4 ppm) atrazine for 20 min. The concentrations of atrazine and its metabolite, hydroxyatrazine, were measured by high-performance liquid chromatography (HPLC).

3. Results and discussion

Blending PVA with a non-electrospinnable material is an effective method to facilitate electrospinning of nanofibers. In polymer solution electrospinning, this method has been employed to facilitate electrospinning of chitosan nanofibers [11]. In the current study, we developed an electrospinning system where the volume ratio of the PVA solution to the TMOS solution and their reaction time can be precisely controlled such that the optimum viscosity of the solution can be maintained throughout the whole electrospinning process, thus allowing continuous formation of silica/PVA nanofibers into reactive mats. The optimized reaction time should be the time it took for the sol to reach an optimum viscosity for electrospinning of uniform nanofibers. Table 1 summarizes the results of electrospun mats fabricated using various combinations of parameters in terms of their fiber diameter (for samples having substantial amount of fibers only), defects, and the corresponding viscosity of the electrospinning solution. Generally, the viscosity of the final solution increased with increasing

Table 1
Parameters investigated for formation of silica-based nanofibers via sol–gel electrospinning coupled with a microfluidic timer and the corresponding results.

Exp.	Concentration of PVA solution [% (w/v)]	PVA-to-TMOS ratio (by volume)	Reaction time (min)	Viscosity of final solution (cP)	Quality in terms of defects[a]	Average fiber diameter (nm)
1	8	1:4	3	687 ± 108	Very poor	N/A
2	8	1:4	9	842 ± 123	Very poor	N/A
3	8	3:4	3	826 ± 116	Very poor	N/A
4	8	3:4	9	1654 ± 257	Poor	109 ± 57
5	18	1:4	3	2113 ± 377	Poor	117 ± 32
6	18	1:4	9	3029 ± 465	Poor	126 ± 33
7	18	3:4	3	2984 ± 447	Good	177 ± 33
8	18	3:4	9	4173 ± 686	Very good	162 ± 82

[a] Very poor quality means that the mat is composed of droplets, polymer blocks, and beaded fibers. Poor quality means that the mat is mainly composed of droplets or beaded fibers with a few non-beaded fibers only. Good quality means that the mat is mainly composed of non-beaded fibers with a few droplets or beads only. Very good quality means that the mat is composed of fibers with no droplets or beaded fibers.

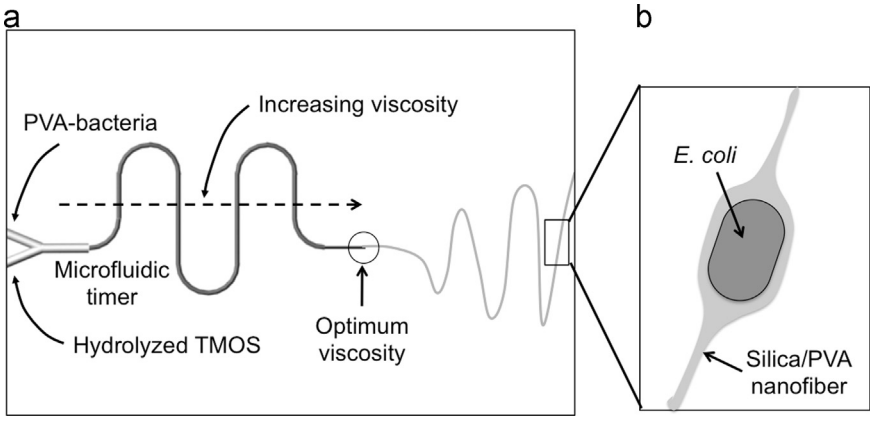

Fig. 1. Formation of silica/PVA biocatalytic nanofibers: (a) microfluidic timer and (b) silica/PVA nanofiber with encapsulated *E. coli*.

concentration of PVA solution, PVA-to-TMOS ratio and reaction time, probably because of the increased degree of crosslinking. As low viscosity caused breaking of the electrospun jet into droplets by the electrostatic force, increasing viscosity could minimize defects such as droplets, polymer blocks or beaded fibers. Fibers with no major defects were obtained at Exp. 8 when the concentration of PVA solution was 18% (w/v) while the PVA-to-TMOS ratio was 0.75 and the reaction time was 9 min. And the average diameter of the fibers fabricated at Exp. 8 was not significantly larger than that of the beaded fibers or fiber with droplets fabricated at Exp. 5, 6 or 7. Therefore, the combination of parameters at Exp. 8 was used for bioencapsulation hereafter.

Silica/PVA hybrid nanofibers with encapsulated *E. coli* bacteria were fabricated via sol–gel electrospinning coupled with the microfluidic timer using the aforementioned optimized parameters. Free GFP expressing *E. coli* bacteria (Fig. 2a) and encapsulated GFP expressing *E. coli* bacteria (Fig. 2b) were examined by fluorescence microscopy. The inset of Fig. 2a shows a free bacterium under TEM. Both free bacteria and encapsulated bacteria were rod-shaped, indicating that the bacteria did not experience a significant morphological change due to bioencapsulation. It can also be clearly seen in Fig. 2b that the encapsulated bacteria were oriented along the longitudinal direction of the nanofiber and they were unevenly distributed. Fig. 2c shows the SEM image of silica/PVA nanofibers with encapsulated bacteria. The surface of the nanofibers was smooth, indicating homogeneous mixing of the PVA with silica. The average diameter of the nanofibers was 182 ± 63 nm, which was similar to the average diameter of the nanofibers obtained at Exp. 8. The inset of Fig. 2c shows an encapsulated *E. coli* bacterium under TEM. Again, it can be clearly seen that the bacterium was rod-shaped and it was aligned along the longitudinal direction of the nanofiber. Fig. 2d shows a photo of an electrospun fabric comprising bacterium-encapsulated nanofibers. The fabric can be made at very large quantities and can be used at industrial scale because of the stabilized solution viscosity throughout the whole electrospinning process.

The FTIR spectra of the silica/PVA nanofibrous mats and the silica/PVA nanofibrous mats with encapsulated *E. coli* bacteria are shown in Fig. 3a. The spectra of both types of nanofibrous mats exhibited several common peaks due to the silica/PVA matrix. These absorption peaks were the scissoring motion of CH_2 at 1430 cm^{-1} and the bending of CH_2 at 1380 cm^{-1}. Apart from the aforementioned common peaks, additional absorption peaks were found in the FTIR spectrum for silica/PVA nanofibrous mats with encapsulated *E. coli* bacteria. This spectrum also exhibited peaks for the Amide I at 1656 cm^{-1} and the Amide II at 1542 cm^{-1}. The peaks for the Amide I and the Amide II confirmed the presence of *E. coli* bacteria in the silica/PVA hybrid nanofibrous mats.

The mass of the encapsulated *E. coli* bacteria in the silica/PVA nanofibers was estimated from the ratio of the intensity of the Amide II peak to the intensity of the CH_2 peak. In this analysis, the Amide II peak, instead of the Amide I peak, was used because the Amide II peak does not include spectral contributions from water bending peak located at 1650 cm^{-1} [12]. The correlation between the Amide II/CH_2 ratio and the mass percentage of *E. coli* bacteria shown in Fig. 3b revealed that at highest capacity using the electrospinning settings described in this letter, the mass percentage of *E. coli* bacteria in the electrospun silica/PVA nanofibrous mat was 44% g/g.

As atrazine is frequently found in aquatic ecosystems, its health and ecosystem effects have been widely studied [13]. Atrazine degradation activity of both free bacteria and encapsulated bacteria were assessed using HPLC analysis. For the encapsulated bacteria, the rate of atrazine degraded or adsorbed (0.57 μmol/g of *E. coli*/min or 0.25 μmol/g of nanofibers/min) was comparable to that of hydroxyatrazine produced (0.56 μmol/g of *E. coli*/min or 0.25 μmol/g of nanofibers/min), implying that adsorption of atrazine into silica was negligible. On the other hand, the specific activity of the encapsulated bacteria (0.57 μmol/g of *E. coli*/min) was comparable to that of the free bacteria (0.64 μmol/g of *E. coli*/min), indicating that the electrospun silica/PVA fibrous mat did not generate significant diffusional resistance.

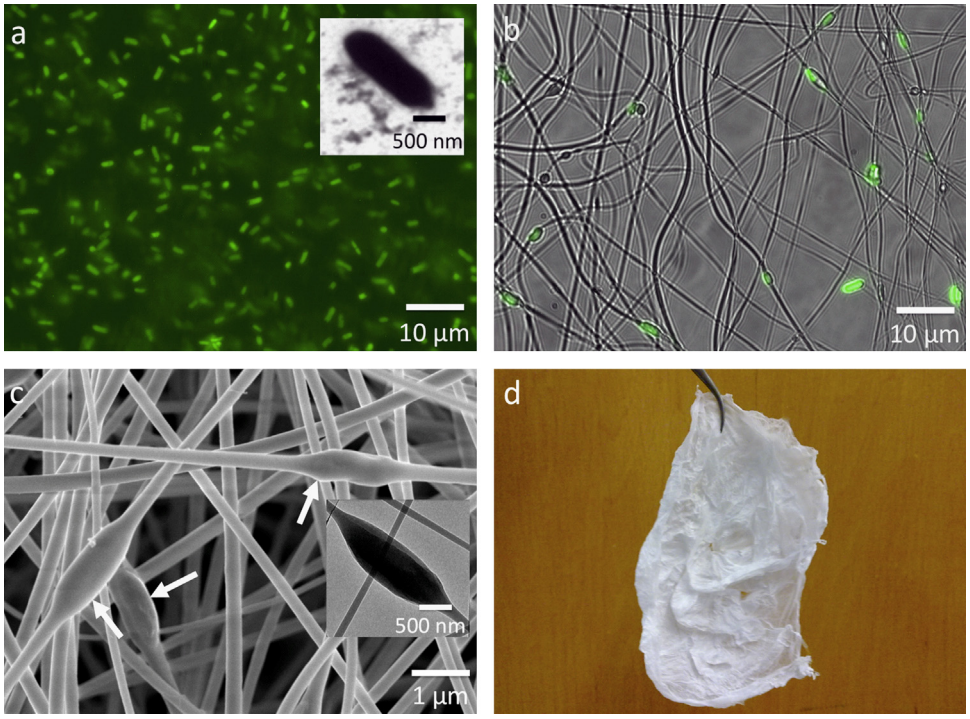

Fig. 2. Free and encapsulated *E. coli*: (a) fluorescence microscopy image of free GFP expressing *E. coli* (inset is a TEM image showing a free bacterium); (b) fluorescence microscopy image of bacteria encapsulated innanofibers; (c) SEM image of silica fiber encapsulated bacteria (white arrows) (inset is a TEM image showing an encapsulated bacterium) and (d) photo of an electrospun fabric comprising bacterium-encapsulated nanofibers.

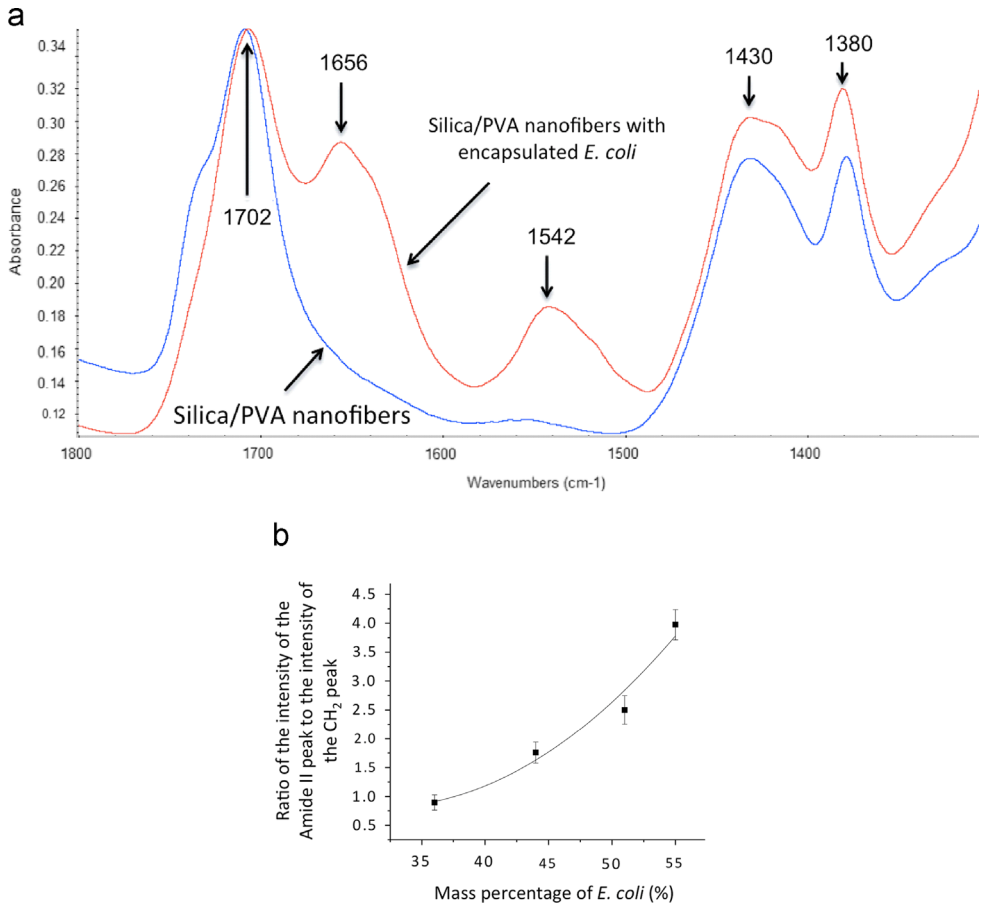

Fig. 3. FTIR analysis: (a) FTIR spectra collected from silica/PVA nanofibers with and without encapsulated *E. coli* and (b) calibration curve for estimating the mass percentage of encapsulated *E. coli* in the electrospun silica/PVA nanofibrous mat.

4. Conclusions

Silica/PVA hybrid nanofibers with encapsulated *E. coli* bacteria were made by sol–gel electrospinning coupled with the microfluidic timer. No organic solvent was used and the electrospun nanofibers were insoluble in water. Up to 44 g of *E. coli* was encapsulated in 100 g of reactive mat. Free-cell levels of atrazine degradation activity could be achieved with encapsulation using the material and the technique developed.

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References

[1] Gill I, Ballesteros A. Trends in Biotechnology 2000;18:282–96.
[2] de-Bashan LE, Bashan Y. Bioresource Technology 2010;101:1611–27.
[3] Zussman E. Polymers for Advanced Technologies 2011;22:366–71.
[4] Liu Y, Rafailovich MH, Malal R, Cohn D, Chidambaram D. Proceedings of the National Academy of Sciences 2009;106:14201–6.
[5] Klein S, Kuhn J, Avrahami R, Tarre S, Beliavski M, Green M, et al. Biomacromolecules 2009;10:1751–6.
[6] Nassif N, Bouvet O, Noelle Rager M, Roux C, Coradin T, Livage J. Nature Materials 2002;1:42–4.
[7] Katoch A, Kim SS. Journal of the American Ceramic Society 2012;95:553–6.
[8] Kim Y-J, Ahn CH, Choi MO. European Polymer Journal 2010;46:1957–65.
[9] Irani M, Keshtkar AR, Moosavian MA. Chemical Engineering Journal 2012;200-202:192–201.
[10] Ponton A, Warlus S, Griesmar P. Journal of Colloid and Interface Science 2002;249:209–16.
[11] Duan B, Wu L, Li X, Yuan X, Li X, Zhang Y, et al. Journal of Biomaterials Science: Polymer Edition 2007;18:95–115.
[12] Pelton JT, McLean LR. Analytical Biochemistry 2000;277:167–76.
[13] Ackerman F. International Journal of Occupational and Environmental Health 2007;13:441–9.