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(54) Title: A METHOD OF ATTACHING A CELL-OF-INTEREST TO A MICROTUBE

(57) Abstract: A method of attaching a cell or a membrane-coated particle-of-interest to a microtube is provided. The method comprising: co-electrospinning two polymeric solutions through co-axial capillaries, wherein a first polymeric solution of the two polymeric solutions is for forming a shell of the microtube and a second polymeric solution of the two polymeric solutions is for forming a coat over an internal surface of the shell, the first polymeric solution is selected solidifying faster than the second polymeric solution and a solvent of the second polymeric solution is selected incapable of dissolving the first polymeric solution and wherein the second polymeric solution comprises the cell or the membrane-coated particle-of-interest, thereby attaching the cell or the membrane-coated particle-of-interest to the microtube. Also provided are microtubes with attached, entrapped or encapsulated cells or membrane-coated particles and methods of using same.



WO 2009/104174 A2

A METHOD OF ATTACHING A CELL-OF-INTEREST TO A MICROTUBE

RELATED APPLICATIONS

5 This application claims priority from U.S. Provisional Application Nos. 61/064,210, 61/064,206 and 61/064,204 filed on February 21, 2008.

 The teachings of PCT/IB2007/054001 are incorporated herein by reference.

 The contents of all of the above documents are incorporated by reference as if fully set forth herein.

10

FIELD AND BACKGROUND OF THE INVENTION

 The present invention, in some embodiments thereof, relates to a method of attaching a cell or a membrane-coated particle-of-interest to a microtube and, more particularly, but not exclusively, to electrospun microtubes which include cells or
15 membrane-coated particles attached, entrapped or encapsulated therein which can be used in various applications such as water purification, detoxification, mineral enrichment, tissue grafts and cell-based therapy.

 Water purification usually entails the removal of toxic chemicals such as mercury, mercurial compounds, and cadmium or elements such as toluene, chloroform,
20 benzene, pesticides and herbicides. Interestingly, although these organic compounds are not found in nature and result from modern industry and motor vehicles, certain bacterial strains have evolved mechanisms for degrading them while utilizing their carbon and nitrogen atoms.

 For example, Pseudomonads can be used to degrade toluene (Moat and Foster,
25 1995), benzene, phenol, naphthalene (Doelle, 1969) and certain hydrocarbons from oil (van der Linden et al., 1965). In addition, atrazine, a commonly used toxic herbicide that enters the water supply, can be detoxified to ammonia and carbon di-oxide via a dechlorination reaction mediated by Pseudomonas ADP. Thus, bioparticles of Pseudomonas ADP grown on granulated active carbon were shown capable of
30 degrading atrazine in water (Herzberg et al., 2006). However, since the granulated active carbon particles are only effective for a limited time, a carbon source (citrate) must be added to the water as it enters the purification column, which may be associated

with the growth of other bacterial species and increases the costs of the purification process.

Another serious problem which occurs in some water systems is the presence of toxic heavy metals such as cadmium and mercury. Several bacterial strains such as Chromobacterium violaceum, Pseudomonas maltophilia, Pseudomonas aeruginosa, Spirulina planensis, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis and Escherichia coli have been found capable of removing metal contamination, destroying the toxic complex containing the metallic ions (e.g., cadmium and tellurium) or recovering valuable metals such as platinum and palladium, gold and silver from the water. For example, certain bacteria have been used to form nanoparticles of valuable metals (Brayner et al., 2007).

Nanofibers and polymeric nanofibers in particular can be produced by the electrospinning process (Reneker DH., et al., 2006; Ramakrishna S., et al., 2005; Li D, et al., 2004; PCT WO 2006/106506 to Zussman, E., et al.). Sun and co-workers (Sun Z, et al., 2003) describe the production of core-shell nanofibers (*i.e.*, filled fibers) by co-electrospinning of two polymeric solutions using a spinneret with two co-axial capillaries. US patent application No. 20060119015 to Wehrspohn R., et al. describes the production of hollow fibers by introducing a liquid containing a polymer to a porous template material, and removal of the template following polymer solidification. PCT/IB2007/054001 to Zussman, E., et al. (which is fully incorporated herein by reference) discloses methods of producing electrospun microtubes (*i.e.*, hollow fibers) which can be further filled with liquids and be used as microfluidics.

Salalha W., et al., 2006, describe the encapsulation of whole bacterial cells and complex bacterial viruses in electrospun single-layer fibers, in which the entrapped cells or viruses retained both physiological activity and some of their viability, even after storing the dry electrospun mats for a number of months.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of attaching a cell or a membrane-coated particle-of-interest to a microtube, the method comprising: co-electrospinning two polymeric solutions through co-axial capillaries, wherein a first polymeric solution of the two polymeric solutions is

for forming a shell of the microtube and a second polymeric solution of the two polymeric solutions is for forming a coat over an internal surface of the shell, the first polymeric solution is selected solidifying faster than the second polymeric solution and a solvent of the second polymeric solution is selected incapable of dissolving the first polymeric solution and wherein the second polymeric solution comprises the cell or the membrane-coated particle-of-interest, thereby attaching the cell or the membrane-coated particle-of-interest to the microtube.

According to an aspect of some embodiments of the present invention there is provided a microtube comprising an electrospun shell, an electrospun coat over an internal surface of the shell and a cell or a membrane-coated particle-of-interest attached to the microtube.

According to an aspect of some embodiments of the present invention there is provided a method of bioremediation, the method comprising contacting a solution containing a contaminant with the microtube of some embodiments of the invention, wherein the cell, a portion of the cell or the membrane-coated particle-of-interest is capable of degrading or assimilating the contaminant.

According to an aspect of some embodiments of the present invention there is provided a method of depleting a molecule from a solution, comprising contacting the solution with the microtube of some embodiments of the invention, wherein the molecule is capable of binding to or being processed by the cell or the membrane-coated particle-of-interest, thereby depleting the molecule from the solution.

According to an aspect of some embodiments of the present invention there is provided a method of isolating a molecule from a solution, comprising: (a) contacting the solution with the microtube of some embodiments of the invention under conditions which allow binding of the molecule to the cell or the membrane-coated particle-of-interest, and; (b) eluting the molecule from the microtube; thereby isolating the molecule from the solution.

According to an aspect of some embodiments of the present invention there is provided a method of detecting a presence of a molecule in a sample, comprising: (a) contacting the sample with the microtube of some embodiments of the invention, wherein the cell or the membrane-coated particle-of-interest is capable of binding to or

processing the molecule, and; (b) detecting the binding or the processing; thereby detecting the presence of the molecule in the sample.

According to some embodiments of the invention, the electrospun shell is formed of a first polymeric solution and the electrospun coat is formed of a second polymeric
5 solution.

According to some embodiments of the invention, the first polymeric solution solidifies faster than the second polymeric solution.

According to some embodiments of the invention, a solvent of the second polymeric solution is incapable of dissolving the first polymeric solution.

10 According to some embodiments of the invention, the electrospun shell comprises a polymer selected from the group consisting of poly (ε-caprolactone) (PCL), polyamide, poly(siloxane), poly(silicone), poly(ethylene), poly(vinyl pyrrolidone), poly(2-hydroxy ethylmethacrylate), poly(N-vinyl pyrrolidone), poly(methyl methacrylate), poly(vinyl alcohol), poly(acrylic acid), poly(vinyl acetate),
15 polyacrylamide, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), poly(methacrylic acid), polylactide, polyglycolide, poly(lactide-coglycolide), polyanhydride, polyorthoester, poly(carbonate), poly(acrylonitrile), poly(ethylene oxide), polyaniline, polyvinyl carbazole, polystyrene, poly(vinyl phenol), polyhydroxyacid, poly(caprolactone), polyanhydride, polyhydroxyalkanoate,
20 polyurethane, collagen, albumin, alginate, chitosan, starch and hyaluronic acid, and whereas the electrospun coat comprises a polymer selected from the group consisting of poly(acrylic acid), poly(vinyl acetate), polyacrylamide, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), poly(methacrylic acid), polylactide polyglycolide, poly(lactide-coglycolide), polyanhydride, polyorthoester, poly(carbonate), poly(ethylene oxide),
25 polyaniline, polyvinyl carbazole, polystyrene, poly(vinyl phenol), polyhydroxyacid, alginate, starch and hyaluronic acid.

According to some embodiments of the invention, a solvent of the first polymeric solution evaporates faster than a solvent of the second polymeric solution.

According to some embodiments of the invention, the electrospinning is effected
30 using a rotating collector.

According to some embodiments of the invention, the solvent of the second polymeric solution is capable of evaporating through the internal surface of the shell.

According to some embodiments of the invention, the second polymeric solution is capable of wetting the internal surface of the shell.

According to some embodiments of the invention, a thickness of the shell is from about 100 nm to about 20 micrometer.

5 According to some embodiments of the invention, an internal diameter of the microtube is from about 50 nm to about 20 micrometer.

According to some embodiments of the invention, the first and the second polymeric solutions are selected from the group consisting of: 10 % poly (ε-caprolactone) (PCL) in chloroform (CHCl₃) and dimethylformamide (DMF) (80:20 by weight) as the first polymeric solution and 4 % poly(ethylene oxide) (PEO) in water (H₂O) and ethanol (60:40 by weight) as the second polymeric solution, 10 % PCL in CHCl₃ and DMF (80:20 by weight) as the first polymeric solution and 6 % PEO in H₂O and ethanol (60:40 by weight) as the second polymeric solution, 9 % PCL in CHCl₃ and DMF (90:10 by weight) as the first polymeric solution and 7 % PEO in H₂O as the second polymeric solution, 10 % PCL in CHCl₃ and DMF (80:20 by weight) as the first polymeric solution and 9 % poly(vinyl alcohol) (PVA) in water and ethanol (50:50 by weight) as the second polymeric solution, and 10 % PCL in CHCl₃ and DMF (90:10 by weight) as the first polymeric solution and 4 % (w/w) PEO in ethanol:H₂O (26:74 by weight) as a second polymeric solution.

20 According to some embodiments of the invention, the microtube is filled with a liquid.

According to some embodiments of the invention, the first and the second polymeric solutions are biocompatible.

25 According to some embodiments of the invention, the cell or the membrane-coated particle-of-interest is attached to the coat over the internal surface of the shell.

According to some embodiments of the invention, the cell or the membrane-coated particle-of-interest is attached to the shell of the microtube.

According to some embodiments of the invention, the first polymeric solution comprises polyethylene glycol (PEG).

30 According to some embodiments of the invention, the shell comprises pores.

According to some embodiments of the invention, the shell prevents diffusion of the cell or the membrane-coated particle-of-interest therethrough.

According to some embodiments of the invention, the cell comprises a prokaryotic cell.

According to some embodiments of the invention, the cell comprises a cell wall.

According to some embodiments of the invention, the contaminant comprises
5 atrazine.

According to some embodiments of the invention, the method further comprising collecting said solution following the contacting.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which
10 the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

15

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and
20 for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-B are images depicting high resolution scanning electron microscope
25 (HRSEM) micrographs of the microtubes according to an embodiment of the invention which are attached to cells of microorganisms such that the cells are entrapped therein. Electrospinning was performed using a first polymeric solution (for forming the shell) which consisted of 9 % [weight/weight (w/w)] polycaprolactone (PCL) dissolved in chloroform/DMSO [9:1 (w/w)]; and a second polymeric solution (for forming the coat
30 over the internal surface of the shell) which consisted of 8 % poly(ethylene oxide) (PEO) in water (w/w). Figure 1A – Magnification of 5000 X; Figure 1B – Magnification of 10,000 X.

FIG. 2 is a microscopic image of *Pseudomonas putida* bacterial cells transformed with the DsRed expression vector encoding the red fluorescent protein [GenBank Accession No. Q9U6Y8 (SEQ ID NO:7)] and entrapped within a microtube of an embodiment of the invention. A microtube was formed by co-electrospinning of the two polymeric solutions: 9 % (w/w) polycaprolactone (PCL) dissolved in chloroform/DMSO [9:1 (w/w)] as a first polymeric solution (for forming the shell); and 8 % poly(ethylene oxide) (PEO) in water (w/w) as second polymeric solution (for forming the coat over the internal surface of the shell) which also included 100 μ l of *Pseudomonas putida* bacterial cells (at a concentration of 10^9 cells/ml). Detection of the bacterial cells within the microtube was performed using a fluorescence microscope. Red fluorescence of the red fluorescent protein (RFP) encoded by the DsRed expression vector was visualized at a wavelength of 359 nm for excitation and examining emitted light at a wavelength of 361 nm. Magnification: 200 X ; Size bar: 20 μ m.

FIGs. 3A-B are schematic illustrations depicting the encapsulation of bacteria (Figure 3A) and the growth of entrapped bacteria (Figure 3B) within the microtube of some embodiments of the invention. Figure 3A – A microtube is formed from two polymeric solutions, the first one, for forming the shell is insoluble in water, and the second one, for forming the coat over the internal surface of the shell is soluble in water (see for example, the description of solutions with Figure 2, hereinabove). Following microtube formation, the microtube can be filled with an aqueous solution (e.g., water or phosphate buffer) by simply exposing the microtube to an aqueous solution (e.g., by immersing the microtube in the aqueous solution). The aqueous solution dissolves some of the polymer of the inner layer (the coat over the internal surface of the shell) which is mixed with the bacterial cells and the bacteria are released to the internal volume of the microtube. The bacteria (red circles) reside within the soluble layer. Figure 3B – The bacteria (red circles) can proliferate within the soluble layer of the microtube when supplied with appropriate nutrients.

FIG. 4 is a schematic illustration of a bioremediation system generated according to some embodiments of the invention. System 300 comprises conduit [220, e.g., part of an aqueous system, can be a plastic or metal (e.g., copper) pipe/tube] having borders (100) includes microtube (230) with shell borders (120) and entrapped bacterial cells (210, red). For purification (e.g., detoxification of water), a liquid (e.g., drinking

water) containing molecule (150, blue) flows within the conduit. Pores (180) within the microtube shell enable the diffusion of molecule (150) through the microtube shell to the microtube lumen (140) containing bacterial cells (210). Cells (210) interact with molecule (150) and reaction product (160, green) diffuses out of the microtube outside of the microtube lumen (140) through the microtube shell pores (180).

FIG. 5 depicts the degradation of atrazine by *Pseudomonas* ADP bacterial cells which are attached to (entrapped or encapsulated within) the microtube of the invention. Atrazine is degraded by the *Pseudomonas* ADP endogenous enzymes: atrazine chlorohydrolase, e.g., GenBank Accession No. NP_862474 (SEQ ID NO:1) encoded by the gene *atzA*, hydroxyatrazine hydrolase, e.g., GenBank Accession No. NP_862481 (SEQ ID NO:2) encoded by *atzB*, N-isopropylammelide isopropylamino hydrolase, e.g., GenBank Accession No. NP_862508 (SEQ ID NO:3) encoded by *atzC*, cyanuric acid amidohydrolase, e.g., GenBank Accession No. NP_862537 (SEQ ID NO:4) encoded by *atzD*, biuret hydrolase, e.g., GenBank Accession No. -NP_862538 (SEQ ID NO:5) encoded by *atzE* and allophanate hydrolase, e.g., GenBank Accession No. AAK50333 (SEQ ID NO:6) encoded by *atzF*.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to methods of attaching a cell or a membrane-coated particle to a microtube and, more particularly, but not exclusively, to microtubes with cells or membrane-coated particles attached, entrapped or encapsulated therein and uses thereof in various purification, bioremediation, isolation, detection, and therapeutic applications.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

While reducing the present invention to practice, the present inventors have devised a method of attaching a cell or a membrane-coated particle to a microtube, to thereby obtain attached, entrapped or encapsulated cells or membrane-coated particles within the microtube.

Thus, as described in Example 1 of the Examples section which follows, the present inventors were capable of attaching cells (e.g., bacterial cells) to a microtube. In addition as shown in Figure 2, cells attached within electrospun microtubes remained intact and viable. Moreover, as shown in Tables 4 and 5 and described in Example 2 of the Examples section which follows, the attached cells preserved their catalytic activity following the electrospinning process and were capable of degrading atrazine from a solution. These results suggest the use of the microtubes of some embodiments of the invention in various applications such a bioremediation of solutions (flow-through applications) and soils, purification, detoxification, and synthesis.

According to one aspect of the invention there is provided a method of attaching a cell or a membrane-coated particle-of-interest to a microtube, the method comprising: co-electrospinning two polymeric solutions through co-axial capillaries, wherein a first polymeric solution of the two polymeric solutions is for forming a shell of the microtube and a second polymeric solution of the two polymeric solutions is for forming a coat over an internal surface of the shell, the first polymeric solution is selected solidifying faster than the second polymeric solution and a solvent of the second polymeric solution is selected incapable of dissolving the first polymeric solution and wherein the second polymeric solution comprises the cell or the membrane-coated particle-of-interest, thereby attaching the cell or the membrane-coated particle-of-interest to the microtube.

As used herein the term "microtube" refers to a hollow tube having an inner diameter from about 50 nm to about 50 μm and an outer diameter from about 0.5 μm to about 100 μm .

According to some embodiments of the invention the inner diameter of the microtube shell of the invention can vary from about 100 nm to about 20 μm , e.g., from about 200 nm to about 10 μm , e.g., from about 500 nm to about 5 μm , e.g., from about 1 μm to about 5 μm , e.g., about 3 μm .

According to some embodiments of the invention the thickness of the microtube shell of the invention can vary from a few nanometers to several micrometers, such as from about 100 nm to about 20 μm , e.g., from about 200 nm to about 10 μm , from about 100 nm to about 5 μm , from about 100 nm to about 1 μm , e.g., about 500 nm.

According to some embodiments of the invention, the microtube may have a length which is from about 0.1 millimeter (mm) to about 20 centimeter (cm), e.g., from about 1-20 cm, e.g., from about 5-10 cm.

As used herein the term "cell" refers to a eukaryotic or prokaryotic cell.

5 According to some embodiments of the invention, the cell-of-interest comprises a cell wall. Non-limiting examples of cells which comprise a cell wall and which can be attached to the microtube of the invention include plant cells, bacteria (e.g., Gram positive and Gram negative bacteria), archaea, protozoa, fungi, and algae.

10 According to some embodiments of the invention, the cell-of-interest comprises a dermis (e.g., insect cells).

According to some embodiments of the invention, the cell has a diameter from about 500 nanometers to about 30 microns, e.g., from about 1-10 microns.

According to some embodiments of the invention, the cell has a diameter from about 1-2 microns.

15 The cell-of-interest which is attached to the microtube may have an activity (e.g., a catalytic activity or a binding activity) which is beneficial (e.g., expression of specific enzymes, e.g., atrazine-degrading enzyme, binding to a specific substrate via a cell receptor or via interaction with a molecule present in the cells, e.g., a DNA, RNA or protein). According to some embodiments of the invention, the cell is genetically
20 modified to express a gene or a protein-of-interest (e.g., a mutant form which is capable of degrading a substrate with an improved catalytic activity as compared to a wild-type form; a specific label, e.g., a green fluorescent protein). Genetic modification can be done using known recombinant DNA technology and include, but not limited to, mutant isolation, know-out or knock-in mutagenesis, site-directed mutagenesis, gene silencing
25 (e.g., siRNA, Ribozyme, DNzyme, antisense) and gene overexpression (e.g., by transfection with an expression vector).

As used herein the phrase "membrane-coated particle" refers to a lipid membrane coated particle. The membrane may enable passage of molecules (e.g., organic and inorganic molecules, polymeric molecules) therethrough.

30 The membrane may be a naturally occurring membrane (e.g., a cell membrane), a portion of a naturally occurring membrane (e.g., a vesicle), an artificial membrane formed of natural membrane components (e.g., a liposome with a lipid bilayer), and/or

an artificial membrane formed of non-natural components such as a polymer, a surfactant [e.g., dioleoylphosphatidylethanolamine (DOPE)], a ceramic, a glass and/or a metal.

Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid
5 crystals, phospholipid dispersions, lamellar layers and the like. The liposomes may be prepared by any of the known methods in the art [Monkkonen, J. *et al.*, 1994, J. Drug Target, 2:299-308; Monkkonen, J. *et al.*, 1993, Calcif. Tissue Int., 53:139-145; Lasic D D., Liposomes Technology Inc., Elsevier, 1993, 63-105. (chapter 3); Winterhalter M, Lasic D D, Chem Phys Lipids, 1993 September;64(1-3):35-43]. The liposomes may be
10 positively charged, neutral or negatively charged. The liposomes may have a single lipid layer or may be multilamellar. If the therapeutic agent is hydrophilic, its delivery may be further improved using large unilamellar vesicles because of their greater internal volume. Conversely, if the therapeutic agent is hydrophobic, its delivery may be further-improved using multilamellar vesicles. Alternatively, the therapeutic agent
15 (e.g. oligonucleotide) may not be able to penetrate the lipid bilayer and consequently would remain adsorbed to the liposome surface. In this case, increasing the surface area of the liposome may further improve delivery of the therapeutic agent. The liposomes can be non-toxic liposomes such as, for example, those prepared from phosphatidylcholine phosphoglycerol, and cholesterol. The diameter of the liposomes used can range
20 from 0.1-1.0 microns.

The particle may comprise an atom, an isotope, a molecule (e.g., a bio-molecule such as an amino acid, a nucleic acid, a polypeptide, a DNA or an RNA), a drug, a virus, a portion of a cell (e.g., a cell vesicle, enzymes of a cell), a bead (e.g., a glass bead, a magnetic bead) or any combination thereof, e.g., a magnetic bead conjugated to a
25 molecule such as a polypeptide, a DNA and/or an RNA.

As used herein the term "attaching" refers to the binding of the cell or the membrane-coated particle-of-interest to the polymer(s) comprised in the microtube of the invention via covalent or non-covalent binding (e.g., via an electrostatic bond, a hydrogen bond, a van-Der Waals interaction) so as to obtain an absorbed, embedded or
30 immobilized cell or membrane-coated particle-of-interest to the microtube of the invention.

According to some embodiments of the invention, the length (L) of the microtube can be several orders of magnitude higher (e.g., 10 times, 100 times, 1000 times, 10,000 times, e.g., 50,000 times) than the microtube's diameter (D). Accordingly, a cell or a membrane-coated particle-of-interest which is attached to a microtube is referred to as being entrapped or encapsulated within the microtube.

According to some embodiments of the invention, covalent attachment of the cell or membrane-coated particle can be via functional groups such as SH groups, amino groups, carboxyl groups which are added to the polymer(s) forming the microtube.

As used herein the phrase "co-electrospinning" refers to a process in which at least two polymeric solutions are electrospun from co-axial capillaries (*i.e.*, at least two capillary dispensers wherein one capillary is placed within the other capillary while sharing a co-axial orientation) forming the spinneret within an electrostatic field in a direction of a collector. The capillary can be, for example, a syringe with a metal needle or a bath provided with one or more capillary apertures from which the polymeric solution can be extruded, e.g., under the action of hydrostatic pressure, mechanical pressure, air pressure and/or high voltage.

The collector serves for collecting the electrospun element (e.g., the electrospun microtube) thereupon. Such a collector can be a rotating collector or a static (non rotating) collector. When a rotating collector is used, such a collector may have a cylindrical shape (e.g., a drum), however, the rotating collector can be also of a planar geometry (e.g., an horizontal disk). The spinneret is typically connected to a source of high voltage, such as of positive polarity, while the collector is grounded, thus forming an electrostatic field between the dispensing capillary (dispenser) and the collector. Alternatively, the spinneret can be grounded while the collector is connected to a source of high voltage, such as with negative polarity. As will be appreciated by one ordinarily skilled in the art, any of the above configurations establishes motion of a positively charged jet from the spinneret to the collector. Reverse polarity for establishing motions of a negatively charged jet from the spinneret to the collector are also contemplated.

For electrospinning, the first polymeric solution is injected into the outer capillary of the co-axial capillaries while the second polymeric solution is injected into the inner capillary of the co-axial capillaries. In order to form a microtube (*i.e.*, a hollow structure, as mentioned above), the first polymeric solution (which is for forming the

shell of the microtube) solidifies faster than the second polymeric solution (also referred herein as a core polymeric solution, and is for forming a coat over the internal surface of the shell). In addition, the formation of a microtube also requires that the solvent of the second polymeric solution be incapable of dissolving the first polymeric solution.

5 The solidification rates of the first and second polymeric solutions are critical for forming the microtube. For example, for a microtube of about 100 μm , the solidification of the first polymer (of the first polymeric solution) can be within about 30 milliseconds (ms) while the solidification of the second polymer (of the second polymeric solution) can be within about 10-20 seconds. The solidification may be a result of polymerization
10 rate and/or evaporation rate.

 According to some embodiments of the invention, the solvent of the first polymeric solution evaporates faster than the solvent of second polymeric solution (e.g., the solvent of the first polymeric solution exhibits a higher vapor pressure than the solvent of the second polymeric solution).

15 According to some embodiments of the invention, the rate of evaporation of the solvent of the first polymeric solution is at least about 10 times faster than that of the solvent of the second polymeric solution. The evaporation rate of the solvent of the first polymeric solution can be at least about 100 times faster or at least about 1000 times faster than the evaporation rate of the solvent of second polymeric solution. For
20 example, the evaporation of chloroform is significantly faster than the evaporation of an aqueous solution (water) due to the high vapor pressure at room temperature of the chloroform (195 mmHg) vs. that of the aqueous solution (23.8 mmHg).

 When selecting a solvent of the second polymeric solution which is incapable of dissolving the first polymeric solution (i.e., a non-solvent of the first polymeric
25 solution), the polymer of the first polymeric solution can solidify (e.g., through precipitation) and form a strong microtube shell which does not collapse, and which is characterized by an even thickness. According to some embodiments of the invention, the first polymeric solution (e.g., the solvent of the first polymer) is substantially immiscible in the solvent of the second polymeric solution.

30 The solvent of the second polymeric solution may evaporate while the polymer (of the second polymeric solution) forms a thin layer on the internal surface of the shell.

According to some embodiments of the invention, the solvent of the second polymeric solution is capable of evaporating through the internal surface of the shell.

The flow rates of the first and second polymeric solutions can determine the microtube outer and inner diameter and thickness of shell. Non-limiting examples are shown in Table 1 hereinbelow.

Table 1
Effect of the flow rates of the two polymeric solutions during electrospinning on microtube diameter and thickness of shell

System No.	System: First polymeric solution/ Second polymeric solution	Flow rates (ml/hr)	R Outer Fiber radius (μm)	d Shell thickness (μm)	V Voltage (kV)	Electrostatic field kV/cm
M5	First polymeric solution	4	3.0-4.5	0.5 $\pm$ 0.1	8.5	0.43
	Second polymeric solution	0.5				
M10	First polymeric solution	10	2.3-4.0	1.0 $\pm$ 0.1	8	0.5
	Second polymeric solution	0.3				
M11	First polymeric solution	10	3-6	1.0 $\pm$ 0.1	9	0.56
	Second polymeric solution	2				

Table 1: Electrospinning was performed with the following solutions: First polymeric solution (for forming the shell) was 10 % PCL in CHCl_3/DMF (8:2 weight/weight); second polymeric solution (for forming the coat) was 4 % PEO in $\text{H}_2\text{O}/\text{EtOH}$ (6:4, weight/weight). PCL 80K; PEO 600K. The temperature during electrospinning was of 22-26 °C. The relative humidity was 58 %, 52 % and 53 % for systems M5, M10 and M11, respectively. The flow rates were measured in milliliter per hour (ml/hr); the outer microtube radius and the shell thickness were measured in microns (μm). The resulting tubes were hollow (good tubes in systems M5 and M11, and mostly good in system M10).

As used herein the phrase “polymeric solution” refers to a soluble polymer, *i.e.*, a liquid medium containing one or more polymers, co-polymers or blends of polymers dissolved in a solvent. The polymer used by the invention can be a natural, synthetic, biocompatible and/or biodegradable polymer.

The phrase "synthetic polymer" refers to polymers that are not found in nature, even if the polymers are made from naturally occurring biomaterials. Examples include, but are not limited to, aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylenes, oxalates, polyamides, tyrosine derived polycarbonates, poly(iminocarbonates), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups, poly(anhydrides), polyphosphazenes, and combinations thereof.

Suitable synthetic polymers for use by the invention can also include biosynthetic polymers based on sequences found in naturally occurring proteins such as those of collagen, elastin, thrombin, fibronectin, or mutant or synthetic derivatives thereof or, starches, poly(amino acids), poly(propylene fumarate), gelatin, alginate, pectin, fibrin, oxidized cellulose, chitin, chitosan, tropoelastin, hyaluronic acid, polyethylene, polyethylene terephthalate, poly(tetrafluoroethylene), polycarbonate, polypropylene and poly(vinyl alcohol), ribonucleic acids, deoxyribonucleic acids, polypeptides, proteins, polysaccharides, polynucleotides and combinations thereof.

The phrase "natural polymer" refers to polymers that are naturally occurring. Non-limiting examples of such polymers include, silk, collagen-based materials, chitosan, hyaluronic acid, albumin, fibrinogen, and alginate.

As used herein, the phrase "co-polymer" refers to a polymer of at least two chemically distinct monomers. Non-limiting examples of co-polymers include, polylactic acid (PLA)-polyethyleneglycol (PEG), polyethylene glycol terephthalate (PEGT) / polybutylene terephthalate (PBT), PLA-polyglycolic acid (PGA), PEG-polycaprolactone (PCL) and PCL-PLA.

As used herein, the phrase "blends of polymers" refers to the result of mixing two or more polymers together to create a new material with different physical properties.

The phrase "biocompatible polymer" refers to any polymer (synthetic or natural) which when in contact with cells, tissues or body fluid of an organism does not induce adverse effects such as immunological reactions and/or rejections, cellular death, and the like. A biocompatible polymer can also be a biodegradable polymer.

According to some embodiments of the invention, the first and the second polymeric solutions are biocompatible.

Non-limiting examples of biocompatible polymers include polyesters (PE), PCL, calcium sulfate, PLA, PGA, PEG, polyvinyl alcohol, polyvinyl pyrrolidone, polytetrafluoroethylene (PTFE, teflon), polypropylene (PP), polyvinylchloride (PVC), polymethylmethacrylate (PMMA), polyamides, segmented polyurethane, polycarbonate-urethane and thermoplastic polyether urethane, silicone-polyether-urethane, silicone-polycarbonate-urethane collagen, PEG-DMA, alginate, hydroxyapatite and chitosan, blends and copolymers thereof.

The phrase "biodegradable polymer" refers to a synthetic or natural polymer which can be degraded (*i.e.*, broken down) in a physiological environment such as by proteases or other enzymes produced by living organisms such as bacteria, fungi, plants and animals. Biodegradability depends on the availability of degradation substrates (*i.e.*, biological materials or portion thereof which are part of the polymer), the presence of biodegrading materials (e.g., microorganisms, enzymes, proteins) and the availability of oxygen (for aerobic organisms, microorganisms or portions thereof), lack of oxygen (for anaerobic organisms, microorganisms or portions thereof) and/or other nutrients. Examples of biodegradable polymers/materials include, but are not limited to, collagen (e.g., collagen I or IV), fibrin, hyaluronic acid, polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), polydioxanone (PDO), trimethylene carbonate (TMC), polyethyleneglycol (PEG), collagen, PEG-DMA, alginate, chitosan copolymers or mixtures thereof.

According to some embodiments, the polymeric solution can be made of one or more polymers, each can be a polymer or a co-polymer such as described hereinabove.

According to some embodiments of the invention, the polymeric solution is a mixture of at least one biocompatible polymer and a co-polymer (either biodegradable or non-biodegradable).

According to some embodiments of the invention, the first polymeric solution for forming the shell can be made of a polymer such as poly (ε-caprolactone) (PCL), polyamide, poly(siloxane), poly(silicone), poly(ethylene), poly(vinyl pyrrolidone), poly(2-hydroxy ethylmethacrylate), poly(N-vinyl pyrrolidone), poly(methyl methacrylate), poly(vinyl alcohol), poly(acrylic acid), poly(vinyl acetate), polyacrylamide, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), poly(methacrylic acid), polylactide, polyglycolide, poly(lactide-coglycolide),

polyanhydride, polyorthoester, poly(carbonate), poly(acrylo nitrile), poly(ethylene oxide), polyaniline, polyvinyl carbazole, polystyrene, poly(vinyl phenol), polyhydroxyacid, poly(caprolactone), polyanhydride, polyhydroxyalkanoate, polyurethane, collagen, albumin, alginate, chitosan, starch, hyaluronic acid, and blends and copolymers thereof.

According to some embodiments of the invention, the second polymeric solution for forming the coat over the internal surface of the shell can be made of a polymer such as poly(acrylic acid), poly(vinyl acetate), polyacrylamide, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), poly(methacrylic acid), polylactide polyglycolide, poly(lactide-coglycolide), polyanhydride, polyorthoester, poly(carbonate), poly(ethylene oxide), polyaniline, polyvinyl carbazole, polystyrene, poly(vinyl phenol), polyhydroxyacid, alginate, starch, hyaluronic acid, and blends and copolymers thereof.

During the formation of the microtube shell (e.g., following the solidification of the first polymeric solution) the second polymeric solution flows within the internal surface of the shell.

According to some embodiments of the invention, the second polymeric solution is selected capable of wetting the internal surface of the shell.

Various polymeric solutions are capable of wetting other polymeric surfaces (for forming the shell). Following is a non-limiting list of pairs of polymeric solutions in which the second polymeric solution is capable of wetting the internal surface of the shell formed by the first polymeric solution.

Table 2
Pairs of polymeric solutions for producing the microtube of the invention

<i>First polymeric solution forming the shell</i>	<i>Second polymeric solution capable of wetting the internal surface of the shell</i>
10 % poly (e-caprolactone) (PCL); in chloroform (CHCl ₃) and dimethylformamide (DMF) (80:20 by weight)	4 % poly(ethylene oxide) (PEO); in water (H ₂ O) and ethanol (60:40 by weight)
Nylon 6,6 in formic acid 7 to 12 wt %	4 % poly(ethylene oxide) (PEO); in water (H ₂ O) and ethanol (60:40 by weight)

<i>First polymeric solution forming the shell</i>	<i>Second polymeric solution capable of wetting the internal surface of the shell</i>
Poly(L-lactide-co-glycolide) (PLGA 10:90) in hexafluoroisopropanol (HFIP) concentrations ranging from 2 to 7 weight % solution.	4 % poly(ethylene oxide) (PEO) in water (H ₂ O) and ethanol (60:40 by weight)
Poly(L-lactide-co-glycolide) (PLGA 15:85) hexafluoroisopropanol (HFIP) concentrations ranging from 2 to 7 weight% solution.	4 % poly(ethylene oxide) (PEO); in water (H ₂ O) and ethanol (60:40 by weight)
poly(lactide-co-glycolide) (PLGA; l-lactide/glycolide _ 50/50) 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) concentrations ranging from 2 to 7 weight% solution.	4 % poly(ethylene oxide) (PEO); in water (H ₂ O) and ethanol (60:40 by weight)
polyglycolide (PGA) in chloroform 3-10 weight % solution.	9 % poly(vinyl alcohol) (PVA); in water and ethanol (50:50 by weight)
poly(L-lactide) (PLA) in chloroform 3-10 weight % solution.	9 % poly(vinyl alcohol) (PVA); in water and ethanol (50:50 by weight)
Segmented polyurethane in DMF and THF (80:20 by weight)	9 % poly(vinyl alcohol) (PVA); in water and ethanol (50:50 by weight)
Polyurethane in DMF and tetrahydrofuran, THF (80:20 by weight)	9 % poly(vinyl alcohol) (PVA); in water and ethanol (50:50 by weight)
PLGA (poly lactic-co-glycolic acid); in chloroform and DMSO (dimethyl sulfoxide) in chloroform and DMSO (80:20 by weight).	9 % poly(vinyl alcohol) (PVA); in water and ethanol (50:50 by weight)
10 % PCL in CHCl ₃ / DMF (80:20 by weight)	6 % PEO in H ₂ O / EtOH (60:40 by weight)
9 % PCL in CHCl ₃ / DMSO (90:10 by weight)	7 % PEO in H ₂ O
10 % PCL in CHCl ₃ / DMF (80:20 by weight)	9 % PVA in ethanol/water (50:50 by weight)
10 % PCL 80 K CHCl ₃ :DMF (90:10 by weight)	4 % (w/w) PEO 600 K; in ethanol:H ₂ O (26:74 by weight)
10 % PCL 80 K +1 % PEG 6 K CHCl ₃ :DMF (90:10 by weight)	4 % (w/w) PEO 600 K; in ethanol:H ₂ O (26:74 by weight)

Table 2 (cont.). The polymers forming the solutions and the solvents are provided by weight ratios, *i.e.*, a weight/weight (w/w) ratio.

According to some embodiments of the invention, the first and the second
5 polymeric solutions are selected from the group consisting of: 10 % poly (ε-caprolactone) (PCL) in chloroform (CHCl₃) and dimethylformamide (DMF) (80:20 by weight) as the first polymeric solution and 4 % poly(ethylene oxide) (PEO) in water (H₂O) and ethanol (60:40 by weight) as the second polymeric solution, 10 % PCL in

CHCl₃ and DMF (80:20 by weight) as the first polymeric solution and 6 % PEO in water and ethanol (60:40 by weight) as the second polymeric solution, 9 % PCL in CHCl₃ and DMF (90:10 by weight) as the first polymeric solution and 7 % PEO in water as the second polymeric solution, 10 % PCL in CHCl₃ and DMF (80:20 by weight) as the first polymeric solution and 9 % poly(vinyl alcohol) (PVA) in water and ethanol (50:50 by weight) as the second polymeric solution and 10 % PCL in CHCl₃ and DMF (90:10 by weight) as the first polymeric solution and 4 % (w/w) PEO in ethanol:water (26:74 by weight) as a second polymeric solution.

According to some embodiments of the invention, the microtube can be filled with a liquid.

To enable a flow of a liquid-of-interest within the microtube, *i.e.*, along the coat polymer covering the internal surface of the shell (which originates from the second polymer solution), the surface (thin film) formed by the coat polymer should be designed such that it can be wetted by the liquid-of-interest. The ability to-wet (wettability) polymer films by liquids is known in the art. For example, silicone oil or water can wet a surface made of a PEO polymer. The wettability of the coat polymer covering the internal surface of the shell can be controlled (e.g., improved) for example by attaching (e.g., using plasma treatment) functional groups such as hydroxyl groups (OH) which increase the hydrophilicity of the coat [see Thurston RM, Clay JD, Schulte MD, Effect of atmospheric plasma treatment on polymer surface energy and adhesion, Journal of Plastic Film & Sheeting 23 (1): 63-78 JAN 2007;which is incorporated within by reference].

For certain applications the microtube shell may comprise pores, thus creating a "breathing" tube. Methods of forming "breathing" microtube (*i.e.*, microtubes with pores in the shell thereof) are described in PCT/IB2007/054001 to Zussman E., et al., which is fully incorporated herein by reference. Briefly, "breathing" tubes can be formed by the inclusion of a high percent (e.g., at least 80 %) of a volatile component such as tetrahydrofuran (THF), chloroform, acetone, or trifluoroethanol (TFE) in the first polymeric solution forming the shell, and/or by the inclusion of a water-soluble polymer such as polyethylene glycol (PEG) in the first polymeric solution forming the shell so that the first polymeric solution comprises a blend of polymers in which one is water-soluble and the other is water-insoluble (e.g., a blend of PEG and PCL).

Alternatively, "breathing" microtubes can be formed by inducing pores in the shell after the completion of the electrospinning process, essentially as described in PCT WO 2006/106506 to the present inventors, which is fully incorporated herein by reference, such as by passing an electrical spark or a heated puncturing element through the electrospun shell, or by using a pulsed or continuous laser beam through the electrospun shell.

According to some embodiments of the invention, the first polymeric solution comprises PEG for inducing pores in the shell. For example, to generate pores greater (>) than 150 nm in diameter, the first polymeric solution may include about 4 % PEG MW 35 kDa. Similarly, to generate pores smaller (<) 150 nm in diameter, the first polymeric solution may include about 2 % PEG MW 6 kDa.

The microtube shell can be designed for selective passage of certain molecules or particles. The passage through the shell pores depends on the size and/or the electrical charge of the molecules/particles with respect to the geometry (length and radius), surface energy, electrical charge of the shell pores, and the viscosity and surface tension of the liquid containing the molecules/particles.

According to some embodiments of the invention, the porosity [i.e., the ratio of the volume of the shell pores to the volume of the shell mass] and pore size can control the release of the cell or the membrane-coated particle-of-interest from the microtube. For example, a shell with pores larger than 1 μm in diameter (e.g., about 1-2 μm) can enable the release of a cell therethrough. In addition, increased porosity can result in a greater rate of release through the shell pores.

Alternatively, the microtube shell can be made such that it prevents diffusion or passage of the cell, the membrane-coated particle-of-interest or any molecule therethrough (e.g., substantially devoid of pores, or with pores having a diameter which is smaller than the cell or the membrane-coated particle-of-interest, or which exhibit a geometry which prevents passage of cells or membrane-coated particles therethrough).

According to some embodiments of the invention, the cell or the membrane-coated particle-of-interest is attached to the polymer of the coat over the internal surface of the shell. For example, as shown in Figure 2 and described in Example 1 of the Examples section which follows, *Pseudomonas putida* cells which express the DsRed fluorescent protein were attached to the internal surface of the shell.

According to some embodiments of the invention, the cell or the membrane-coated particle is attached to the microtube shell.

According to some embodiments of the invention attachment of the cell (e.g., a eukaryotic cell such as a mammalian cell) or the membrane-coated particle is performed following microtube formation. For example, the microtube can be soaked with a solution containing the cell/membrane-coated particle. The cell/membrane-coated particle can diffuse through the shell pores and enter the inner lumen of the microtube. In addition, the microtube can be covalently attached to the cell/membrane-coated particle (e.g., via SH groups).

Regardless of the method of production, the present invention provides a microtube which comprises an electrospun shell, an electrospun coat over an internal surface of the shell and a cell or a membrane-coated particle-of-interest attached to the microtube.

As used herein, the phrase “electrospun shell” refers to a hollow element of a tubular shape, made of one or more polymers, produced by the process of electrospinning as detailed above.

As used herein the phrase “electrospun coat” refers to a thin layer covering the internal surface of the shell of the microtube of the invention which is made of one or more polymers by the process of electrospinning as detailed above.

One of ordinary skill in the art will know how to distinguish an electrospun object from objects made by means which do not comprise electrospinning by the high orientation of the macromolecules, the skin (e.g., shell) morphology, and the typical dimensions of the microtube which are unique to electrospinning.

The microtube of the invention can form an individual (e.g., single or separated) microtube or can form part of a plurality (e.g., an aligned array) of microtubes which can be either connected to each other or separated (as single, not-connected microtubes).

For the production of a single microtube a fork like clip is attached to the edge of the rotating disk. The disk is rotated for 1-2 seconds and individual microtubes are formed between the sides of the clip. In a similar way individual electrospun fibers were collected [see E. Zussman, M. Burman, A.L. Yarin, R. Khalfin, Y. Cohen, “Tensile Deformation of Electrospun Nylon 6,6 Nanofibers,” *Journal of Polymer*

Science Part B: Polymer Physics, 44, 1482-1489, (2006), herein incorporated by reference in its entirety].

Alternatively, when using a rotating collector, a plurality of microtubes can be formed and collected on the edge of the collector as described elsewhere for electrospun
5 fibers [A. Theron, E. Zussman, A.L. Yarin, "Electrostatic field-assisted alignment of electrospun nanofibers", *Nanotechnology J.*, 12, 3: 384-390, (2001); herein incorporated by reference in its entirety].

The plurality of microtubes can be arranged on a single layer, or alternatively, the plurality of microtubes define a plurality of layers hence form a three dimensional
10 structure. The microtubes can have a general random orientation, or a preferred orientation, as desired. For example, when the fibers are collected on a cylindrical collector such as a drum, the microtubes can be aligned predominantly axially or predominantly circumferentially. Different layers of the electrospun microtubes can have different orientation characteristics. For example, without limiting the scope of the
15 present invention to any specific ordering or number of layers, the microtubes of a first layer can have a first predominant orientation, the microtubes of a second layer can have a second predominant orientation, and the microtubes of third layer can have general random orientation.

The microtube of the invention can be available as a dry fibrous mat(s) (e.g., as
20 spun dry microtubes) or as a wetted mat(s) (e.g., following immersing or filling the microtube with a liquid).

According to some embodiments of the invention, the microtube which is attached to the cell or the membrane-coated particle-of-interest is configured as or in a microfluidics device. "Lab-on-a-chip" is described in a series of review articles [see for
25 example, Craighead, H. "Future lab-on-a-chip technologies for interrogating individual molecules". *Nature* 442, 387-393 (2006); deMello, A. J. "Control and detection of chemical reactions in microfluidic systems". *Nature* 442, 394-402 (2006); El-Ali, J., Sorger, P. K. & Jensen, K. F. "Cells on chips". *Nature* 442, 403-411 (2006); Janasek, D., Franzke, J. & Manz, A. "Scaling and the design of miniaturized chemical-analysis
30 systems". *Nature* 442, 374-380 (2006); Psaltis, D., Quake, S. R. & Yang, C. H. "Developing optofluidic technology through the fusion of microfluidics and optics". *Nature* 442, 381-386 (2006); Whitesides, G. M. "The origins and the future of

microfluidics". Nature 442, 368-373 (2006); Yager, P. et al. "Microfluidic diagnostic technologies for global public health". Nature 442, 412-418 (2006)] each of which is fully incorporated herein by reference].

According to some embodiments of the invention, the liquid which fills in, flows
5 in or surrounds the microtube enables the desorption (detachment) of the cell or the membrane-coated particle from the microtube (e.g., from the polymer included in the coat over the internal surface of the shell). According to of some embodiments of the invention the desorption process facilitates the interaction between the entrapped or encapsulated cell/membrane-coated particle with a molecule-of-interest (e.g., a substrate
10 of an enzyme contained within the cell/membrane coated particle). According to some embodiments of the invention, the desorption process enables the flow and/or the release of the cell/membrane-coated particle within and from the microtube.

The cell or the membrane-coated particle-of-interest which is attached, entrapped or encapsulated within the microtube of the invention can be either an intact
15 cell (i.e., having an un-ruptured membrane/cell wall) or non-intact cell (i.e., with a ruptured membrane/cell wall).

According to some embodiments of the invention, the cell or the membrane-coated particle-of-interest which is attached to, entrapped or encapsulated within the microtube of the invention maintains the activity, or at least a portion thereof, which it
20 possessed prior to the attachment (e.g., of the same cell or the membrane-coated particle-of-interest prior to the electrospinning process, or when unattached to the microtube). For example, a bacterial cell with a ruptured cell wall/membrane may still contain the enzymatic activity of its proteins.

The term "activity" as used herein refers to any of a catalytic activity, kinetics,
25 and/or affinity to a substrate or a ligand which the cell or the membrane-coated particle may have. Such an activity can be any biological activity such as catalysis, binding (with a specific affinity), hybridization, chelation, degradation, synthesis, catabolism, hydrolysis, polymerization, transcription, drug activity and the like.

As used herein the phrase "at least a portion of the activity" refers to at least
30 about 10 %, at least about 20-50 %, e.g., more than about 50 %, e.g., more than about 60 %, e.g., more than about 70 %, e.g., more than about 75 %, e.g., more than about 80

%, e.g., more than about 90 %, e.g., more than about 95 % of the activity which the cell or the membrane-coated particle possessed prior to the attachment to the microtube.

For example, as mentioned before and described in the Examples section which follows, the bacterial cells entrapped within the microtube of some embodiments of the invention preserved the specific activity to their substrates (atrazine).

The microtube of the invention with the attached, entrapped or encapsulated active cell or the membrane-coated particle-of-interest can be used in various applications which require the attachment of active cells (including portions thereof) or membrane-coated particles to a support and optionally also the controlled release therefrom.

According to some embodiments of the invention, the microtube of the invention is attached to more than one type of cells/membrane-coated particles. The microtube can be attached to a mixture of cells from several species or from a single species. The combination of cells can be selected according to the intended use. For example, several cells which are involved in complex reactions (e.g., processing of a substrate or a mixture of substrates) can be used.

According to some embodiments of the invention the microtube can be used as a micro-reactor (e.g., bioreactor) for chemical transition reactions [e.g., a multi-step reaction (cascade)] requiring high concentrations of several enzymes, e.g., enzymes produced by a certain cell (e.g., a bacterial cell) or by several cells or several different species (e.g., several types of bacterial cells).

According to an aspect of the invention, there is provided a method of processing a substrate-of-interest. The method is effected by contacting the substrate-of-interest with the microtube of the invention, wherein the cell or the membrane-coated particle which is attached to, entrapped or encapsulated within the microtube is capable of processing the substrate, thereby processing the substrate-of-interest.

As used herein the term "processing" refers to a catalytic activity performed by the cell or the membrane-coated particle which is attached to, entrapped or encapsulated within the microtube on its cognate substrate.

According to some embodiments of the invention processing involves enzymatic-dependent conversion (catalysis) of a substrate from a given chemical form to a distinct one. Examples of such catalysis reactions include, but are not limited to

degradation, digestion, hydrolysis, nucleic acid cleavage, nucleic acid ligation, proteolytic cleavage, polymerization, transfer of an atom or functional group from one molecule to another and addition of a chemical group to a molecule.

According to some embodiments of the invention, such a process simply
5 incorporates (endocytose) the substrate-of-interest such as for use in a reaction (synthesis) catalyzed by the cell or the membrane-coated particle.

According to some embodiments of the invention, the microtube can be used for the synthesis of rare biochemicals such as intermediates in biosynthesis pathways which are normally present at very low intracellular concentrations. For example, for the
10 synthesis of indole glycerol phosphate the microtube according to some embodiments of the invention may be attached to a cell which expresses the enzymes participating in indole glycerol phosphate synthesis. For example, to accumulate indole glycerol phosphate the cell can be a mutant cell lacking the enzyme (or having a non-functional enzyme) converting indole glycerol phosphate to indole.

15 According to some embodiments of the invention, such a process can be the incorporation of the substrate-of-interest in a catabolism reaction catalyzed by the cell or membrane-coated particle which is attached, entrapped or encapsulated within the microtube.

For example, the catabolism reaction can be the degradation (e.g., by hydrolysis)
20 of a toxic molecule for the purpose of detoxification (e.g., detoxifying water) or decomposition of an unwanted molecule.

According to an aspect of the invention there is provided a method of depleting a molecule from a composition containing the molecule, the method is effected by contacting the composition with the microtube of the invention, wherein the molecule is
25 capable of binding to or being processed by the cell or the membrane-coated particle-of-interest, thereby depleting the molecule from the composition.

The composition containing the molecule may be in a liquid form (e.g., a solution), a solid form (e.g., soil) or a gel form. The molecule can be mixed with the composition or bound to the composition (by covalent or non-covalent bindings).

30 According to some embodiments of the invention, the method further comprising collecting the composition following the contacting.

As used herein the term “depleting” refers to removing an amount e.g., at least about 50 %, at least about 60 %, at least about 70 %, at least about 80 %, at least about 90 %, at least about 95 %, e.g., 99 %, e.g., 100 % of the molecule from the composition.

According to some embodiments of the invention, the depletion (removal) of the molecule from the composition is effected within a short time period, such as within
5 minutes, hours or several days (e.g., about 1-7 days)

As used herein the phrase “contacting” refers to enabling the interaction between the molecule and the cell or the membrane-coated particle-of-interest which is attached to, entrapped or encapsulated within the microtube, for a time period which is sufficient
10 for depleting the molecule from the composition (e.g., solution, soil). Such a contact can take place, for example, while the composition (e.g., solution) is passing through (e.g., via capillary forces) the end(s) of the hollow structure of the microtube and/or through the shell pores. Additionally or alternatively, such a contact between the molecule and the cell or the membrane-coated particle-of-interest can take place by incubating the
15 microtube in the composition (e.g., by placing the microtube in a container including a solution, a soil or a gel)).

The solution can be a water-based or an oil-based solution which includes inorganic or organic molecules, such as a biological sample or a sample from a non-living source such as stream, industrial waste or ocean waters. As used herein the
20 phrase “biological sample” refers to any sample derived from a living organism such as plant, bacteria or animals, and can include cells or alternatively be cell-free (i.e., include only a biological fluid).

According to some embodiments of the invention, the solution is an aqueous solution such as drinking water, groundwater and/or industrial waste water. According
25 to some embodiments of the invention, the microtube of the invention forms part of an aqueous system designed for treatment of the aqueous solution (e.g., for depleting, eliminating or removing toxic moieties therefrom).

According to some embodiments of the invention, depleting is effected via bioremediation (i.e., the process of biodegradation and/or assimilation of a contaminant
30 by microorganisms, fungi, green plants or their enzymes). Bioremediation can be used to remove contaminants such as soil contaminants, chlorinated hydrocarbons, crude oil herbicides, heavy metals, and the like.

According to some embodiments of the invention, depleting is effected via biodegradation.

As used herein the term "biodegradation" refers to degradation of a substrate using bio-molecules (e.g., enzymes) which are expressed by (or contained within) the
5 cell or the membrane-coated particle which is attached to the microtube of the invention.

For example, to remove petroleum pollutants [e.g., aliphatics (e.g., C5-C36) and aromatics (e.g., C9-C22) such as benzene, toluene, ethylbenzene and xylenes (BTEx), phenol, naphthalene or certain hydrocarbons from oil] from water (biodegradation of petroleum pollutants), the microtube of the invention may be attached to bacteria
10 utilizing alkanes as a sole source of carbon and energy or to membrane-coated particles including alkane-degrading enzymes. Such alkanes can be, for example, methane, ethane, propane, butane and mixtures thereof).

Non-limiting examples of butane-utilizing bacteria include Gram negative and Gram positive aerobic rods and cocci, facultative anaerobic Gram negative rods, non-
15 photosynthetic, non-fruiting gliding bacteria and irregular non-sporing Gram positive rods. Of the Pseudomonadaceae family comprising Gram-negative aerobic rods and cocci, species of the following genera may be suitable: Pseudomonas; Variovorax; Chryseobacterium; Comamonas; Acidovorax; Stenotrophomonas; Sphingobacterium; Xanthomonas; Frateuria; Zoogloea; Alcaligenes; Flavobacterium; Derxia;
20 Lampropedia; Brucella; Xanthobacter; Thermus; Thermomicrobium; Halomonas; Alteromonas; Serpens; Janthinobacterium; Bordetella; Paracoccus; Beijerinckia; and Francisella. Of the Nocardioform Actinomycetes family comprising Gram-positive Eubacteria and Actinomycetes, the following genera may be suitable: Nocardia; Rhodococcus; Gordona; Nocardioides; Saccharopolyspora; Micropolyspora;
25 Promicromonospora; Intraspangium; Pseudonocardia; and Oerskovia. Of the Micrococcaceae family comprising Gram-positive cocci, the following genera may be suitable: Micrococcus; Stomatococcus; Planococcus; Staphylococcus; Aerococcus; Peptococcus; Peptostreptococcus; Coprococcus; Gemella; Pediococcus; Leuconostoc; Ruminococcus; Sarcina; and Streptococcus. Of the Vibrionaceae family comprising
30 facultative anaerobic Gram-negative rods, the following genera may be suitable: Aeromonas; Photobacterium; Vibrio; Plesiomonas; Zymomonas; Chromobacterium; Cardiobacterium; Calymmatobacterium; Streptobacillus; Eikenella; and Gardnerella.

Of the Rhizobiaceae family comprising Gram-negative aerobic rods and cocci, the following genera may be suitable: *Phyllobacterium*; *Rhizobium*; *Bradyrhizobium*; and *Agrobacterium*. Of the Cytophagaceae family comprising non-photosynthetic, non-fruiting, gliding bacteria, the following genera may be suitable: *Cytophaga*; *Flexibacter*;
5 *Saprospira*; *Flexithrix*; *Herpetosiphon*; *Capnocytophaga*; and *Sporocytophaga*. Of the *Corynebacterium* family comprising irregular, non-sporing Gram-positive rods, the following genera may be suitable: *Aureobacterium*; *Agromyces*; *Arachnia*; *Rothia*; *Acetobacterium*; *Actinomyces*; *Arthrobacteria*; *Arcanobacterium*; *Lachnospira*; *Propionibacterium*; *Eubacterium*; *Butyrivibria*; *Brevibacterium*; *Bifidobacterium*;
10 *Microbacterium*; *Caseobacter*; and *Thermoanaerobacter*.

Non-limiting examples of methane-utilizing bacteria include *Methylomonas* (e.g., *Methylomonas albus* such as the BG 8 strain; *Methylomonas methanica* such as the PM strain), *Methylobacter* (e.g., *Methylobacterium organophilum*), *Methylococcus* [e.g., *Methylococcus capsulatus*, such as the Texas strain ATCC 19069 and the Bath
15 strain National Collection of Industrial Bacteria (NCIB) 11132], *Methylocystis* (*Methylocystis parvus*), and *Methylosinus* (e.g., *Methylosinus trichosporium* such as the OB 3b strain, e.g., NCIB No. 11131 and The Fermentation Research Institute (FRI), Japan (as FERM-P4981)].

For example, for the biodegradation of cyanide [depletion of cyanide from a
20 solution such as industrial waste water (e.g., of silver mining)], bacterial cells or membrane-coated particles capable of degrading free (CN^- or HCN) or complexed (e.g., metal-cyanide complex) cyanide can be attached to the microtube of the invention. For example, degradation of free cyanide can be performed using the archaee bacterium *Sulfolobus* which is also used in gold extraction from low grade minerals [see Knowles,
25 C.J. and Bunch, A.W., "Microbial cyanide metabolism. Advances in Microbial Physiology", (1986); 27: 73 111]; Complexed cyanide such as silver-cyanide [$\text{Ag}(\text{CN})_2$] can be degraded using the *Citrobacter* sp. MCM B-181, *Pseudomonas* sp. MCM B-182, *Pseudomonas* sp. MCM B-183 and *Pseudomonas* sp. MCM B-184 bacteria [see Patil YB, Paknikar KM, Letters in Applied Microbiology, (2000), 30: 33–37] which utilize
30 metal–cyanide complexes as a nitrogen source and release ammonia and carbon dioxide as degradation products.

To remove toxic moieties of herbicides that enters the water supply, such as the chlorine entity of atrazine (biodegradation of atrazine), a microtube which includes cells or membrane-coated particles (e.g., portions of cells, liposomes) capable of degrading atrazine can be used. For example, bacterial cells or portions of cells including the atrazine degrading enzymes: atrazine chlorohydrolase, hydroxyatrazine hydrolase, N-isopropylammelide isopropylamino hydrolase, cyanuric acid amidohydrolase, biuret hydrolase, and allophanate hydrolase (Figure 5) can be used. Such bacteria can be the *Pseudomonas ADP* (which endogenously express the ATZ genes) or any other bacterial strain which exogenously express the ATZ genes (*atzA-atzF* genes), such as the *Pseudomonas putida* S12 described in the Examples section which follows.

For example, as described in Tables 4 and 5 and Example 2 of the Examples section which follows, efficient atrazine degradation was achieved by microtubes which were attached to the *Pseudomonas ADP* or *Pseudomonas putida* S12 that express the *atzA-atzF* genes.

According to some embodiments of the invention, biodegradation of atrazine is effected such that more than about 50 %, e.g., more than about 60 %, e.g., more than about 70 %, e.g., more than about 80 % of atrazine is removed from a solution containing about 20 mg atrazine per liter following 1-4 days of contacting the solution with the microtube. According to exemplary embodiments of the invention, biodegradation of atrazine is effected such that more than about 90 %, more than about 95 %, e.g., 100 % of atrazine is removed from a solution containing about 20 mg atrazine per liter following 1-2 days of contacting the solution with the microtube. ,

As mentioned hereinabove, the microtube of the invention may form part of an aqueous system designed for treatment of the aqueous solution.

Figure 4 schematically illustrates a single configuration of a bioremediation system generated according to some embodiments of the invention. System 300 comprises conduit [220, e.g., part of an aqueous system, can be a plastic or metal (e.g., copper) pipe/tube] having borders (100) includes microtube (230) with shell borders (120) and entrapped bacterial cells (210). For purification (e.g., detoxification of water), a liquid (e.g., drinking water) containing molecule (150) flows within the conduit. Pores (180) within the microtube shell enable the diffusion of molecule (150) through the microtube shell to the microtube lumen (140) containing bacterial cells

(210). Cells (210) interact with molecule (150) and reaction product (160) diffuses out of the microtube outside of the microtube lumen (140) through the microtube shell pores (180).

According to some embodiments of the invention, a microtube with entrapped
5 bacterial cells expressing the atrazine degrading enzymes is placed (or packed within) a column [e.g., conduit (220) as shown in Figure 4] filled with an aqueous solution (e.g., drinking water). The small diameter of the microtube (e.g., 3-5 μm) and the significant length (e.g., 20 cm) provides an enormous surface area for degrading atrazine. As mentioned above, due to the dimensions of the microtube (wherein the length is several
10 orders of magnitude larger than the diameter), the cells are entrapped within the microtube and are not washed away. Accordingly, there is no need to add a carbon source to the bacterial cells.

According to some embodiments of the invention, the cells entrapped within the microtube can continue to degrade atrazine for an extended time period without the
15 formation of a biofilm (i.e., a complex aggregation of microorganisms marked by the excretion of a matrix). According to some embodiments of the invention, the microtube shell prevents passage of predators such as Bdellovibrios, protozoa, and bacteriophage thus protecting the entrapped bacterial cells from such predators.

According to some embodiments of the invention, the bacterial cells may be
20 refreshed by immersing the microtube in growth medium which contains usable carbon, nitrogen, phosphorus and sulfur sources and thereby both allowing the cells to proliferate and/or renew their metabolic potential.

According an aspect of the invention, there is provided a method of isolating a molecule from a solution. The method is effected by (a) contacting the solution with
25 the microtube of the invention under conditions which allow binding of the molecule to the cell or the membrane-coated particle-of-interest, and; (b) eluting the molecule from the microtube; thereby isolating the molecule from the solution.

As used herein the term "isolating" refers to physically separating the molecule from the solution or its other components by binding the molecule to the cell or
30 membrane-coated particle which is attached to, entrapped or encapsulated within the microtube and eluting the bound molecule therefrom. As used herein the term "eluting" refers to dissociating the bound molecule from the microtube. Those skilled in the art

are capable of adjusting the conditions required for eluting (e.g., releasing) the molecule from the microtube and/or separating the molecule from the cell or membrane-coated particle.

According to an aspect of the invention, there is provided a method of detecting a presence of a molecule in a sample. The method is effected by (a) contacting the sample with the microtube of the invention, wherein the cell or the membrane-coated particle-of-interest is capable of binding to or processing the molecule, and (b) detecting the binding or the processing; thereby detecting the presence of the molecule in the sample.

As used herein the phrase "detecting binding or the processing" refers to identifying a change in the concentration, conformation, spectrum or electrical charge of the molecule in the sample and/or of the cell or membrane-coated particle that is attached to the microtube following the binding therebetween or following the processing of the molecule by the cell or the membrane-coated particle. Identification of the binding or processing can be performed using methods known in the art such as following the fluorescence or the color of the sample, radioactivity of the sample, the electrical conductivity of the sample and the like. Binding of a molecule to a cell may be via a specific receptor on the cell.

According to some embodiments of the invention, the cell or the membrane-coated particle-of-interest which is attached to the microtube is labeled or comprises a label [e.g., by genetic modification as described above, or by conjugation to a dye, fluorophore, radio-isotope, or an enzyme (e.g., horse radish peroxidase) capable of producing a colorimetric product], and detecting the binding or processing of a molecule is performed by following such a label.

The microtube according to some embodiments of the invention can be used as a biosensor, for the detection of molecules in a sample. Such a biosensor can be advantageous over known open field biosensors (e.g., sensors in which the cell or membrane-coated particle is conjugated to a solid support not having a tubular structure, such as a flat support) especially due to the increased ratio between the size of the microtube surface (which attaches the cell/membrane-coated particle) and the volume of the sample being in contact therewith.

The microtube according to some embodiments of the invention can be used to release the attached cell or membrane-coated particle through pores in the shell or the microtube openings (i.e., the ends of the tubular structure). According to some embodiments of the invention, the microtube can be placed in a supercritical fluid (e.g., liquid nitrogen) for a short period of time (e.g., 2-10 minutes), following which the microtube is cut (e.g., with a sharp knife, razor blade) close to its end. The microtube can be also subject to cross linking using, e.g., glutaraldehyde which assists in preserving the openness of the microtube.

The microtube according to some embodiments of the invention can be attached to bacteria that produce and contain nano-magnets. Such bacteria were discovered and isolated more than 30 years ago (Blakemore, R 1975 Magnetotactic bacteria. Science 190:377-379; Bayzliniski, D.A., Frankel, R.B. and Jannasch, H.W. 1989 Anaerobic magnetite production by marine, magnetotactic bacteria. Nature 334:518-519). Some of these strains contain a string of magnets whose poles, N and S, are aligned. Without being bound by any theory, during the electrospinning process and the flow of the second polymeric solution the bacteria can be oriented such that their long axis is parallel to that of the microtube (see an example of rod bacteria aligned within the microtube; Figure 2). When using bacteria that contain magnets, the bacteria can be aligned by applying magnetic force and their tendency to be aligned by flow such that the attached bacteria are aligned in a polar manner where all their N's face in the same direction.).

Microtubes with attached magnet producing bacteria can be used in industry (e.g., microelectronics) and medical applications (such as imaging).

The microtube according to some embodiments of the invention can be used in medical dialysis to remove materials from blood and other bodily fluids such as urine.

The microtube of some embodiments the invention can be used in various *ex vivo* and *in vivo* applications. For example, the microtube can be attached to cells (e.g., mammalian) capable of inducing tissue formation [e.g., stem cells such as adult stem cells (tissue stem cells) or embryonic stem cells]. Briefly, the microtube can be contacted with a medium (e.g., tissue culture medium, physiological solution, blood) which is suitable for proliferation, differentiation and/or migration of the cell so as to enable tissue formation.

The microtube of some embodiments of the invention (e.g., a microtube made of biocompatible polymers) can be implanted in a subject in need thereof.

As used herein the phrase a “subject in need thereof” refers to any animal subject e.g., a mammal, e.g., a human being which suffers from a pathology (disease, disorder or
5 condition) which can be treated by the cell/membrane coated particle which is attached, entrapped or encapsulated within the microtube of the invention.

The term “treating” as used herein refers to inhibiting, preventing or arresting the development of a pathology and/or causing the reduction, remission, or regression of a pathology. Those of skill in the art will understand that various methodologies and
10 assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology.

According to some embodiments of the invention, the microtube is implanted in a subject to induce *in vivo* formation of a tissue.

15 According to some embodiments of the invention, the microtube directs the attached (or entrapped) cell or membrane-coated particle-of-interest (e.g., a drug in a liposome) to a target tissue of a subject (e.g., targeted delivery of a drug).

Methods of implanting grafts such as the microtube of the invention into a subject are known in the art. For example, the microtube can be implanted
20 subcutaneously, intradermally, or into any body cavity (e.g., abdomen), as well as into the vascular system (using e.g., a hollow catheter delivery system). Alternatively, the microtube of the invention can be connected to a body conduit (e.g., a blood vessel such as a vein or an artery) such that it enables the flow of a fluid therethrough.

The invention further envisages the use of the microtube of the invention, which
25 includes a cell or a membrane-coated particle-of-interest attached thereto, for guiding cell growth *ex vivo* or *in vivo*. For example, neuronal cells which are attached or entrapped within the microtube can be contacted with solution containing growth factors and/or nutrients needed for neuronal growth. It will be appreciated that once an initial neuronal growth has occurred *ex vivo*, such a system (*i.e.*, the microtube with the
30 neuronal cells) can be implanted in a subject in need thereof (e.g., a subject with degenerated, damaged or injured neuronal cells) to thereby enable neuronal growth and guidance.

The microtube of some embodiments of the invention can be included in a kit/article of manufacture along with a packaging material and/or instructions for use in any of the above described methods or applications.

The methods described herein may be conducted batchwise.

5 It will be appreciated that the microtubes of the some embodiments of the present invention can find wide use in waste, commodity, food, agrotec, cosmetic and pharma industries. A detailed discussion of some embodiments is not meant to be limiting.

As used herein the term "about" refers to $\pm 10\%$.

10 The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics
15 of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be
20 presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such
25 as from 1 to 6 should be considered to have specifically disclosed sub ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited
30 numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein

interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat.

Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

GENERAL MATERIALS AND EXPERIMENTAL METHODS

Electrospinning solutions: The compositions of the shell and core solutions are given in Table 3, hereinbelow. All polymers and solvents were purchased from Sigma-Aldrich and were used as is.

Bacterial cells and growth medium - *Pseudomonas putida* S12 (exogenously expressing atz genes by transfection with a plasmid including the coding sequences of the atz genes depicted in Figure 5) and *Pseudomonas* ADP (which endogenously express the atz genes), both of which are capable of degrading atrazine and using it as a source of nitrogen, were grown to stationary phase in an ATZ culture medium (modified from Mandelbaum R. T., Wackett L. P., Allan D. L., 1993. "Mineralization of the s-triazine ring of atrazine by stable bacterial mixed cultures". Applied and Environmental Microbiology 59: 1695-1701) in which atrazine was included as the sole source of nitrogen. The ATZ culture medium was prepared as follows: For each liter of double distilled water the following were added: KH_2PO_4 1.6 grams, K_2HPO_4 0.4 grams, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 grams, NaCl 1 gram, CaCl_2 25 mg and citrate (sodium-citrate) 2 g; 20 mg atrazine; 20 ml of a vitamin and trace element stock solution. The vitamin and trace element stock solution contains per liter: Nitrilotriacetic acid 10 grams, KOH 7.3 grams, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 14.45 grams, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 9.25 grams, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 100 mg, nicotinic acid 50 mg, biotin 0.5 mg, thiamin HCl 2.5 mg, and 44 metal solution 50 ml. 44 metal solution contains per 100 ml: EDTA 250 mg, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 1.1 grams, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 grams, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 154 mg, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 50 mg, $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ 75 mg, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ 20 mg.

The bacterial cells (10^9 cells/ml) grown in the above medium were added to the core solution (e.g., PEO in water or PEO + 40 % ethanol) (e.g., at a ratio of 9:1 (volume/volume) core solution to cells) and electrospinning was performed using the shell and core solutions shown in Table 3, hereinbelow, so as to form a microtube in which the bacterial cells are attached to the internal surface (coat) of the shell. The presence and localization of the bacterial cells following formation of the microtube was determined using a fluorescence microscope.

Table 3
Two types of core-shell microtubes: composition of the solutions

Type	Shell solution	Core solution
1	10 % PCL 80 K; in CHCl ₃ :DMF (90:10 by weight)	4 % (w/w) PEO 600 K in ethanol:H ₂ O (26:74 by weight) and bacterial cells (10 ⁹ cells/ml) in a 10:1 ratio (900 µl of polymer solution and 100 µl of bacterial cell)
1	9 % PCL 80 K; in CHCl ₃ :DMSO (90:10 by weight)	8 % (w/w) PEO 600 K in water + bacterial cells (10 ⁹ cells/ml) in a 10:1 ratio (900 µl of polymer solution and 100 µl of bacterial cell)
2	10 % PCL 80 K +1 % PEG 6 K; in CHCl ₃ :DMF (90:10 by weight)	4 % (w/w) PEO 600 K in ethanol:H ₂ O (26:74 by weight) + bacterial cells (10 ⁹ cells/ml) in a 10:1 ratio (900 µl of polymer solution and 100 µl of bacterial cell)

Table 3. Microtubes were formed by co-electrospinning of the shell solution (a first polymeric solution for forming the shell) and a core solution (a second polymeric solution for forming the coat over the internal surface of the shell). Type 1 microtubes - do not include PEG in the shell solution; Type 2 microtubes - include PEG in the shell solution.

Electrospinning – Hollow microtubes (core-shell hollow fibers) were fabricated by a co-electrospinning process using the set up described by Sun et al. (2003) and Zussman et al. (2006) with the polymeric solutions (for forming the shell and coat over the internal surface of the shell) as described in Table 3 above. All experiments were conducted at room temperature (about 22 °C) and a relative humidity of about 35 %. The spinning parameters were as follow: 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/hour for the shell solution and 1 ml/hour for the core solution. The fibers were collected as a strip on the edge of a vertical rotating wheel (Theron A., et al., (2001)) having a velocity of 1.2 m/second. 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 wave lengths of 359 and 361 nm, respectively, was used for the imaging of fibers filled with fluorescent product.

5 **Measurement of Atrazine degradation** - The mat containing the entrapped bacterial cells was immersed in a medium [ATZ-containing phosphate buffer (20 mg Atrazine per liter of phosphate buffer)] and following predetermined time periods (e.g., 1 to 6 days) aliquots from the medium surrounding the mat(s) were taken and the amount of atrazine was measured using HPLC. The aliquots were mixed with an equal
10 volume of methanol and were centrifuged for 10 minutes at 10,000 X g to remove salts and other insoluble materials. High- performance liquid chromatography (HPLC) was conducted on Hewlett Packard HPLC (HP110 series). Separation was done at a flow rate of 0.6 ml/minute on a LiChrospher 100 RP-18 (5 μ m) column (LiChroCART HPLC Cartridge system 250-4; Merck, Darmstadt, Germany), using 200 mM
15 $\text{NH}_4\text{CH}_3\text{COO}$ in 70:30 % [volume/volume (v/v)] methanol/water as a mobile phase. Concentration of atrazine was quantified relative to authentic standard by integrating peak area at 220 nm.

20

EXAMPLE 1

ENTRAPMENT OF BACTERIAL CELLS WITHIN MICROTUBES

Experimental Results

Attachment of bacterial cells within an electrospun microtube - A microtube with cells entrapped therein was formed by co-electrospinning of the two polymeric
25 solutions: 9 % [weight/weight (w/w)] polycaprolactone (PCL) dissolved in chloroform/DMSO (9:1; w/w) as a first polymeric solution (for forming the shell); and 8 % poly(ethylene oxide) (PEO) in water as second polymeric solution (for forming the coat over the internal surface of the shell) which also included *Pseudomonas putida* or
30 *Pseudomonas* ADP bacterial cells (at a concentration of 10^9 cells/ml) at a ratio of 9:1 [volume/volume (v/v); 900 μ l polymeric solution and 100 μ l of bacterial cells]. The *Pseudomonas putida* S12 cells had been previously transformed to express the DsRed fluorescent protein (GenBank Accession No. Q9U6Y8, SEQ ID NO:7), such that

following microtube formation, the red fluorescence originating from the *Pseudomonas putida* cells can be observed using a fluorescence microscope. As is shown in Figure 2, the *Pseudomonas putida* cells were entrapped within the electrospun microtube. In addition, the observed red fluorescence demonstrates that the cells were intact.

5

EXAMPLE 2

ATRAZINE DEGRADATION USING BACTERIAL CELLS ENTRAPPED WITHIN THE MICROTUBE

Experimental Results

10 ***Degradation of atrazine using bacterial cells which are entrapped within the microtube of the invention*** - *Pseudomonas putida* S12 or *Pseudomonas* ADP, both of which are capable of degrading atrazine and using it as a source of nitrogen, were grown to stationary phase in a medium in which atrazine was included as the sole source of nitrogen. The cells were added to the core solution [e.g., 4 % PEO in 40 %
15 ethanol (w/w); or 8 % PEO in water (w/w)] and electrospun with the shell-forming solution (without the addition of PEG to the shell solution) to render microtubes with a core-shell structure. The microtubes (2 types for each strain) were tested for their ability to degrade atrazine. The microtubes were immersed in a flask containing phosphate buffer with atrazine (20 mg atrazine in 1 liter of phosphate buffer) and
20 degradation was followed by examining the amount of residual atrazine using HPLC (conducted on Hewlett Packard HPLC, HP110 series), in aliquots taken at predetermined time periods (from one day to several days). The microtubes were then transferred to a new flask and the procedure repeated.

25 The percentages of atrazine removal following incubation of the *Pseudomonas* ADP cells – containing microtubes in ATZ-containing phosphate buffer are summarized in Table 4, hereinbelow.

Table 4
Degradation of atrazine by electrospun microtubes
containing *Pseudomonas* ADP

<i>Days from beginning of experiment</i>	<i>Transfer No.</i>	<i>Incubation time in ATZ culture medium (days) before transfer</i>	<i>% Atrazine removal using microtubes with solution A as core</i>	<i>% Atrazine removal using microtubes with solution B as core</i>
0	1	1	59.0	32.3
1	2	2	51.8	20.8
3	3	3	87.9	94.0
6	4	1	76.5	91.5
7	5	2	91.2	94.3
14	6	6	85.0	85.0
15	7	1	96.0	96.0
16	8	1	94.2	94.5
17	9	3	100.0	100.0
20	10	1	100.0	100.0
21	11	2	97.5	97.5

Table 4: The degradation of atrazine by electrospun microtubes containing *Pseudomonas* ADP bacterial cells. Shown are the percentages of atrazine removal following predetermined incubation periods of microtubes with entrapped *Pseudomonas* ADP bacterial cells in a phosphate buffer which contains ATZ (20 mg atrazine per liter). The core solutions used for forming the microtube coat over the internal surface of the shell were: solution A - 4 % PEO in 40 % ethanol; and solution B - 8 % PEO in water.

As shown in Table 4, hereinabove, when a microtube which was prepared with a core solution containing 4 % PEO in 40 % ethanol was used, efficient removal of atrazine (e.g., about 95 % removal) was observed throughout the experiment and the entrapped bacterial cells within the microtubes continued to remove atrazine even after 11 transfers. When a microtube which was prepared with a core solution containing 8 % PEO in water was used, efficient removal of atrazine (e.g., about 95 % removal) was observed throughout the time of the experiment and the microtube preparation (with entrapped cells) remained competent in this regard even after 21 days and 11 transfers.

Microtubes with attached cells were incubated ATZ growth medium (for 4 days, 30 °C), and then the microtubes were transferred to ATZ-containing phosphate buffer for the beginning of experiment. .

The percentages of atrazine removal following incubation of the *Pseudomonas putida* S12 bacterial cells - containing microtubes in an ATZ-containing phosphate buffer are summarized in Table 5, hereinbelow.

Table 5
Degradation of atrazine by electrospun microtubes
containing *Pseudomonas putida* S12

<i>Days from beginning of experiment</i>	<i>Transfer No.</i>	<i>Incubation time in ATZ culture medium (days) before transfer</i>	<i>% Atrazine removal using microtubes with 5 % PEO in water as a core solution</i>
0	1	4	100
4	2	3	100
7	3	2	100
9	4	2	100
11	5	3	100
16	6	2	98.3
21	7	5	100

Table 5: The degradation of atrazine by electrospun microtubes containing *Pseudomonas putida* S12 bacterial cells. Shown are the percentages of atrazine removal following predetermined incubation periods of microtubes with entrapped S12 bacterial cells in a phosphate buffer which contains ATZ (20 mg atrazine per liter). The core solution used for forming the microtube coat over the internal surface of the shell was 5 % PEO in water.

As shown in Table 5, hereinabove, 100 % removal of atrazine from the solution was observed following 4 days incubation of the S12-containing microtubes of the invention in the ATZ-containing phosphate buffer. Efficient removal of atrazine continued through seven transfers and remained complete in microtube material to the 21st day.

This is a new and efficient way to remove atrazine from water in e.g., a continuous flow system composed of columns filled with electrospun hollowfibers containing suitable bacterial cells (e.g., which are capable of degrading atrazine). Thus, the influent water which contains atrazine penetrates (e.g., by diffusion) into the hollow fibers (the microtubes) and the atrazine is broken down to non-toxic material, such that the effluent water is free of atrazine.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all

such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

5 All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

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WHAT IS CLAIMED IS:

1. A method of attaching a cell or a membrane-coated particle-of-interest to a microtube, the method comprising: co-electrospinning two polymeric solutions through co-axial capillaries, wherein a first polymeric solution of said two polymeric solutions is for forming a shell of the microtube and a second polymeric solution of said two polymeric solutions is for forming a coat over an internal surface of said shell, said first polymeric solution is selected solidifying faster than said second polymeric solution and a solvent of said second polymeric solution is selected incapable of dissolving said first polymeric solution and wherein said second polymeric solution comprises the cell or the membrane-coated particle-of-interest, thereby attaching the cell or the membrane-coated particle-of-interest to the microtube.

2. A microtube comprising an electrospun shell, an electrospun coat over an internal surface of said shell and a cell or a membrane-coated particle-of-interest attached to the microtube.

3. The microtube of claim 2, wherein said electrospun shell is formed of a first polymeric solution and said electrospun coat is formed of a second polymeric solution.

4. The microtube of claim 3, wherein said first polymeric solution solidifies faster than said second polymeric solution.

5. The microtube of claim 3, wherein a solvent of said second polymeric solution is incapable of dissolving said first polymeric solution.

6. The microtube of claim 2, wherein said electrospun shell comprises a polymer selected from the group consisting of poly (ε-caprolactone) (PCL), polyamide, poly(siloxane), poly(silicone), poly(ethylene), poly(vinyl pyrrolidone), poly(2-hydroxy ethylmethacrylate), poly(N-vinyl pyrrolidone), poly(methyl methacrylate), poly(vinyl alcohol), poly(acrylic acid), poly(vinyl acetate), polyacrylamide, poly(ethylene-co-vinyl

acetate), poly(ethylene glycol), poly(methacrylic acid), polylactide, polyglycolide, poly(lactide-coglycolide), polyanhydride, polyorthoester, poly(carbonate), poly(acrylonitrile), poly(ethylene oxide), polyaniline, polyvinyl carbazole, polystyrene, poly(vinyl phenol), polyhydroxyacid, poly(caprolactone), polyanhydride, polyhydroxyalkanoate, polyurethane, collagen, albumin, alginate, chitosan, starch, hyaluronic acid, and whereas said electrospun coat comprises a polymer selected from the group consisting of poly(acrylic acid), poly(vinyl acetate), polyacrylamide, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), poly(methacrylic acid), polylactide polyglycolide, poly(lactide-coglycolide), polyanhydride, polyorthoester, poly(carbonate), poly(ethylene oxide), polyaniline, polyvinyl carbazole, polystyrene, poly(vinyl phenol), polyhydroxyacid, alginate, starch, hyaluronic acid.

7. The method of claim 1 or the microtube of claim 3, 4 or 5, wherein a solvent of said first polymeric solution evaporates faster than a solvent of said second polymeric solution.

8. The method of claim 1, wherein said electrospinning is effected using a rotating collector.

9. The method of claim 1 or the microtube of claim 3, 4 or 5, wherein a solvent of said second polymeric solution is capable of evaporating through said internal surface of said shell.

10. The method of claim 1 or the microtube of claim 3, 4 or 5, wherein said second polymeric solution is capable of wetting said internal surface of said shell.

11. The method of claim 1 or the microtube of claim 2, wherein a thickness of said shell is from about 100 nm to about 20 micrometer.

12. The method of claim 1 or the microtube of claim 2, wherein an internal diameter of the microtube is from about 50 nm to about 20 micrometer.

13. The method of claim 1 or the microtube of claim 3, 4 or 5, wherein said first and said second polymeric solutions are selected from the group consisting of: 10 % poly (ε-caprolactone) (PCL) in chloroform (CHCl₃) and dimethylformamide (DMF) (80:20 by weight) as said first polymeric solution and 4 % poly(ethylene oxide) (PEO) in water (H₂O) and ethanol (60:40 by weight) as said second polymeric solution, 10 % PCL in CHCl₃ and DMF (80:20 by weight) as said first polymeric solution and 6 % PEO in H₂O and ethanol (60:40 by weight) as said second polymeric solution, 9 % PCL in CHCl₃ and DMF (90:10 by weight) as said first polymeric solution and 7 % PEO in H₂O as said second polymeric solution, 10 % PCL in CHCl₃ and DMF (80:20 by weight) as said first polymeric solution and 9 % poly(vinyl alcohol) (PVA) in water and ethanol (50:50 by weight) as said second polymeric solution, and 10 % PCL in CHCl₃ and DMF (90:10 by weight) as the first polymeric solution and 4 % (by weight) PEO in ethanol:H₂O (26:74 by weight) as a second polymeric solution.

14. The method of claim 1 or the microtube of claim 2, 3, 4 or 5, wherein said microtube is filled with a liquid.

15. The method of claim 1 or the microtube of claim 3, 4 or 5, wherein said first and said second polymeric solutions are biocompatible.

16. The method of any of claims 1, 7-15, or the microtube of any of claims 2-7, 9-15, wherein said cell or said membrane-coated particle-of-interest is attached to said coat over said internal surface of said shell.

17. The method of any of claims 1, 7-15 or the microtube of any of claims 2-7, 9-15, wherein said cell or said membrane-coated particle-of-interest is attached to said shell of the microtube.

18. The method of any of claims 1, 7-17 or the microtube of any of claims 3-7, 9-17, wherein said first polymeric solution comprises polyethylene glycol (PEG).

19. The method of any of claims 1, 7-18 or the microtube of any of claims 2-7, 9-18, wherein said shell comprises pores.

20. The method of any of claims 1, 7-17 or the microtube of any of claims 2-7, 9-17, wherein said shell prevents diffusion of the cell or the membrane-coated particle-of-interest therethrough.

21. The method of any of claim 1, 7-20 or the microtube of any of claims 2-7, 9-20, wherein said cell comprises a prokaryotic cell.

22. The method of any of claims 1, 7-21 or the microtube of any of claims 2-7, 9-21, wherein said cell comprises a cell wall.

23. A method of bioremediation, the method comprising contacting a solution containing a contaminant with the microtube of any of claims 2-7, 9-20, wherein said cell, a portion of said cell or said membrane-coated particle-of-interest is capable of degrading or assimilating said contaminant.

24. The method of claim 23, wherein said contaminant comprises atrazine.

25. A method of depleting a molecule from a solution, comprising contacting the solution with the microtube of any of claims 2-7, 9-20, wherein the molecule is capable of binding to or being processed by said cell or said membrane-coated particle-of-interest, thereby depleting the molecule from the solution.

26. The method of claim 23, 24 or 25, further comprising collecting said solution following said contacting.

27. A method of isolating a molecule from a solution, comprising:

(a) contacting the solution with the microtube of any of claims 2-7, 9-20 under conditions which allow binding of the molecule to said cell or said membrane-coated particle-of-interest, and;

- (b) eluting the molecule from the microtube;
thereby isolating the molecule from the solution.

28. A method of detecting a presence of a molecule in a sample, comprising:

- (a) contacting the sample with the microtube of any of claims 2-7, 9-20,
wherein said cell or said membrane-coated particle-of-interest is capable of binding to
or processing said molecule, and;

- (b) detecting said binding or said processing;
thereby detecting the presence of the molecule in the sample.

1/5



FIG. 1B

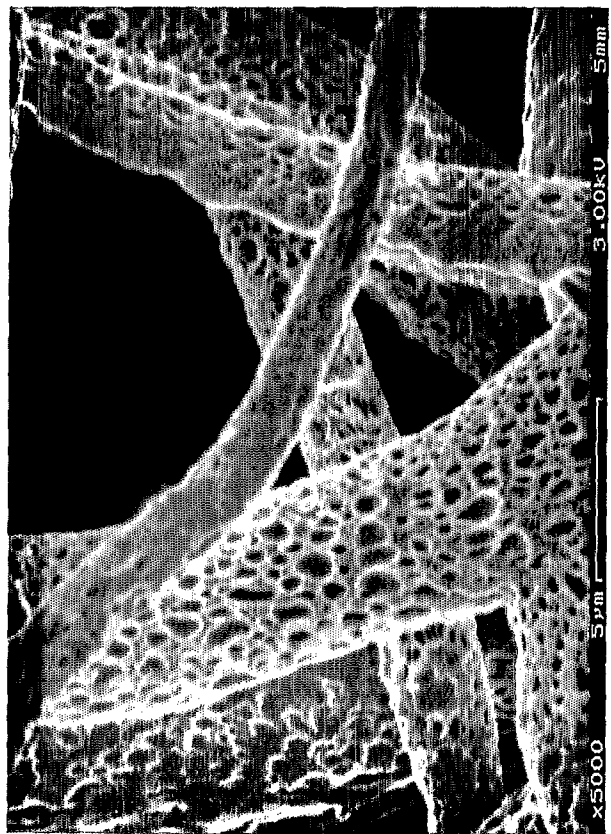


FIG. 1A

2/5

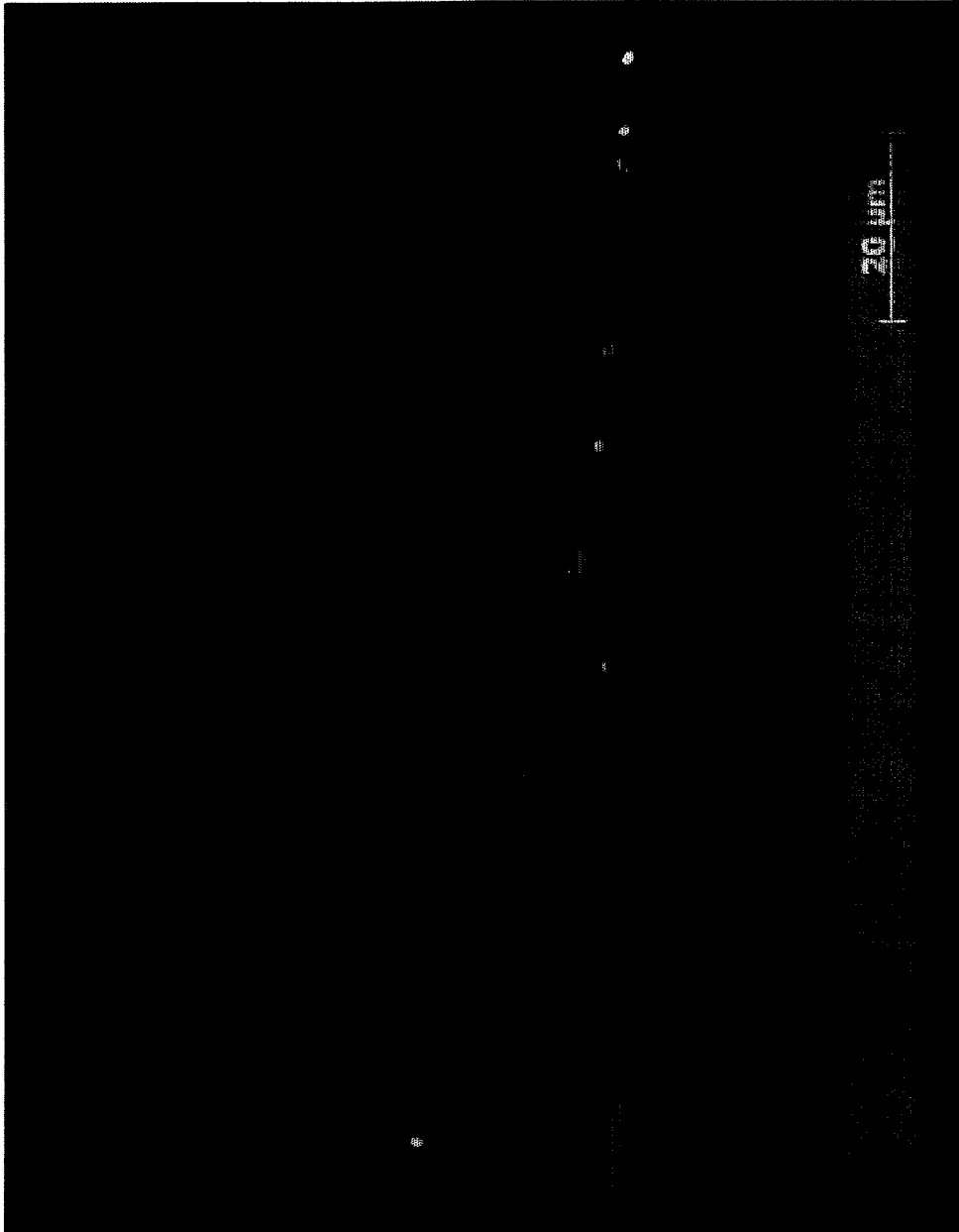


FIG. 2

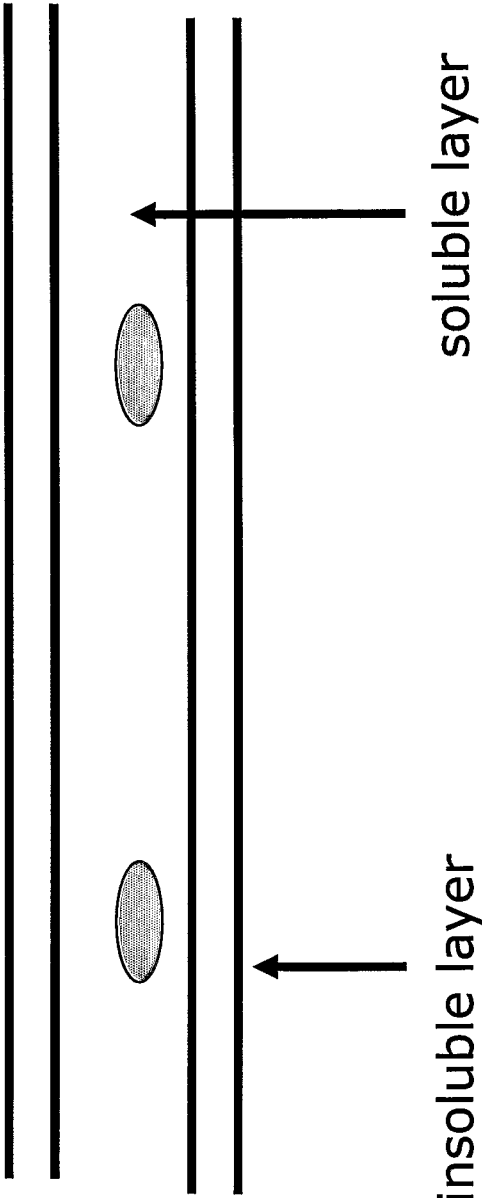


FIG. 3A

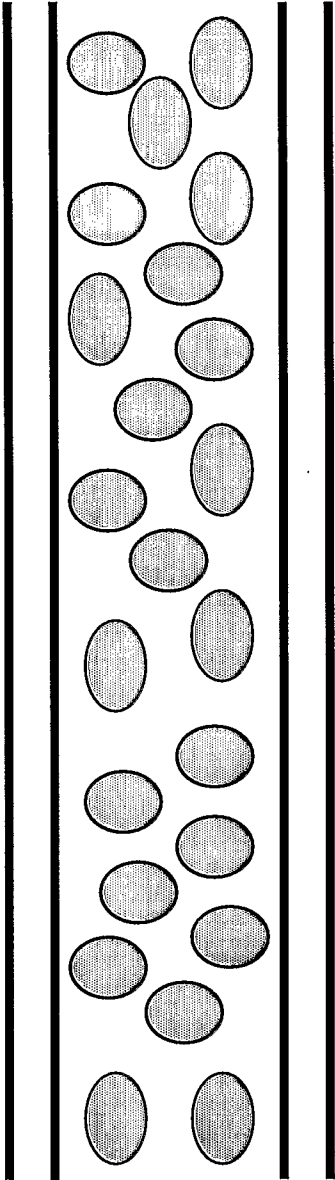


FIG. 3B

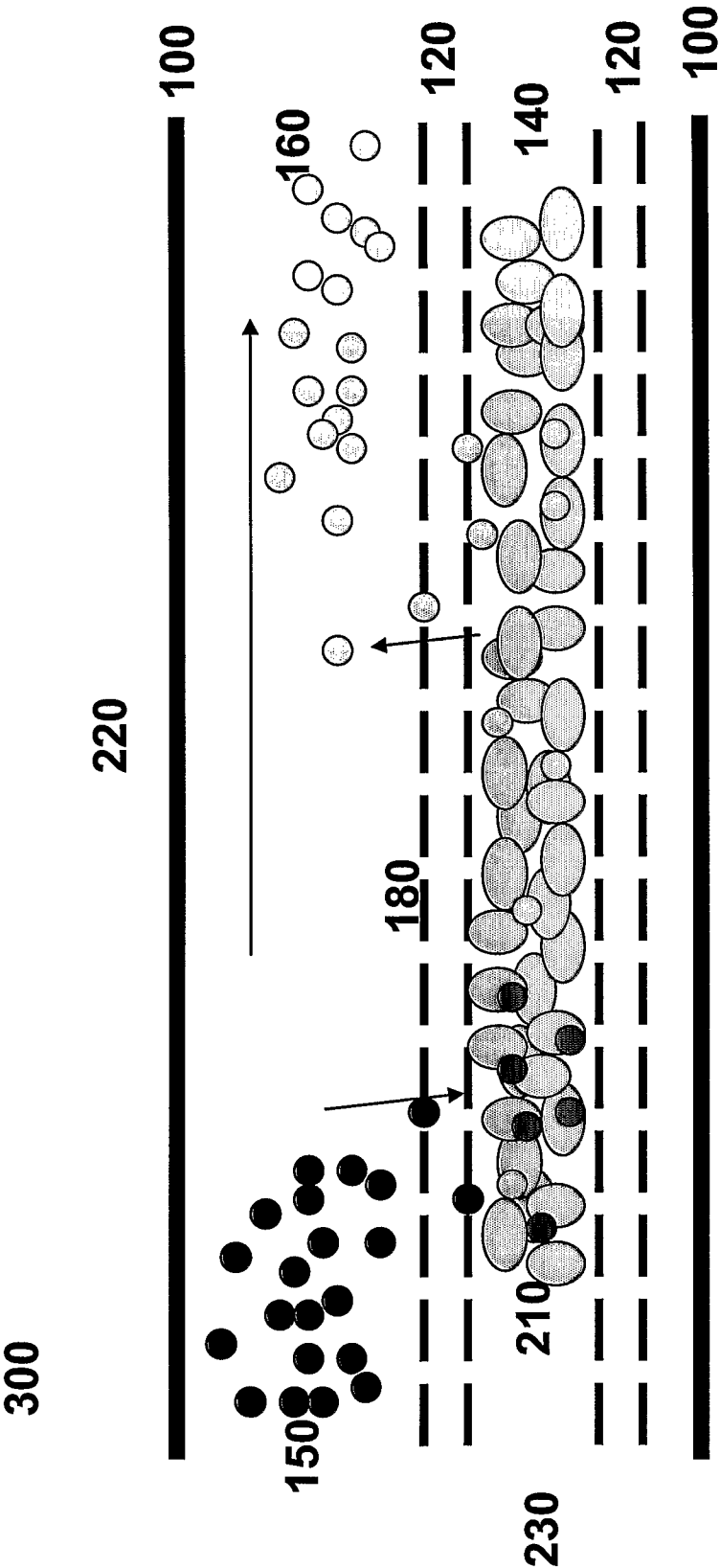


FIG. 4

5/5

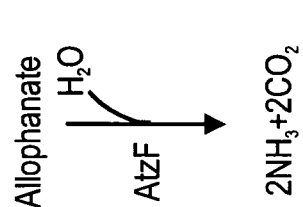
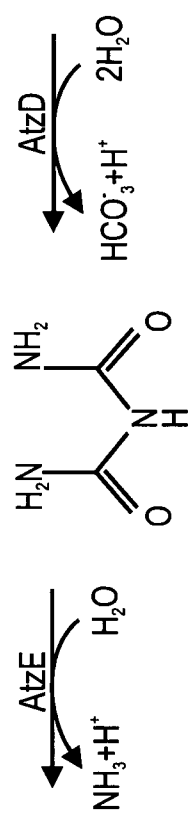
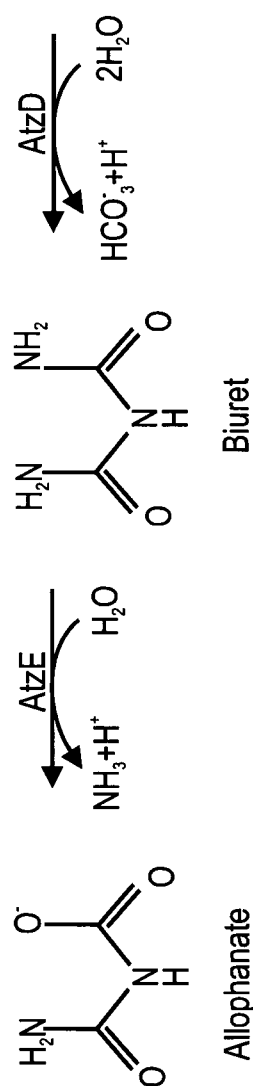
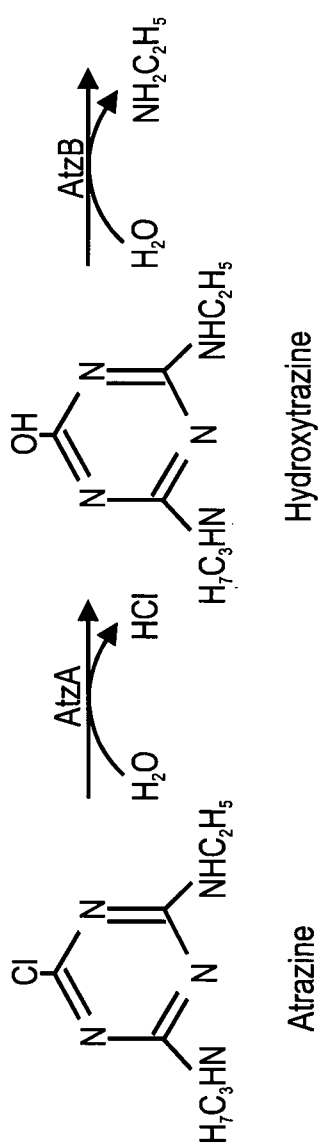
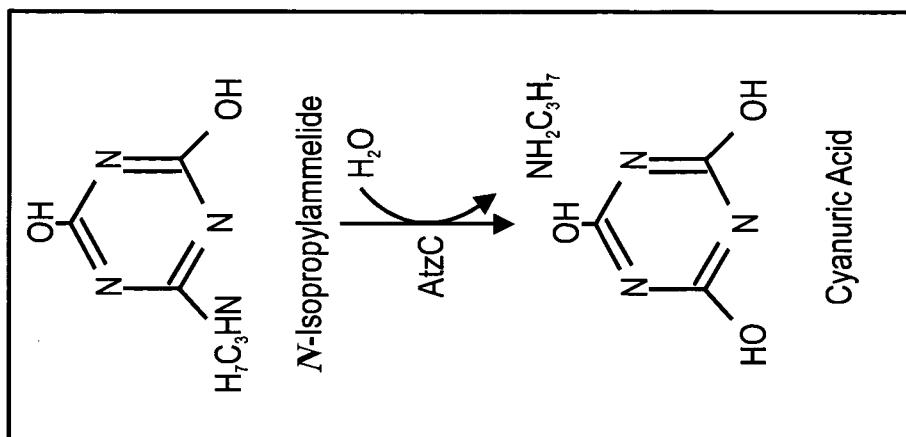


FIG. 5