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(54) Title: METHODS OF ATTACHING A MOLECULE-OF-INTEREST TO A MICROTUBE

(57) Abstract: Provided is a method of attaching a molecule-of-interest to a microtube, by 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 molecule-of-interest, thereby attaching the molecule-of-interest to the microtube. Also provided is an electrospun microtube comprising an electrospun shell, an electrospun coat over an internal surface of the shell and a molecule-of-interest attached to the microtube.



WO 2009/104176 A2

METHODS OF ATTACHING A MOLECULE-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 invention, in some embodiments thereof, relates to a method of attaching a molecule-of-interest to a microtube and, more particularly, but not exclusively, to electrospun microtubes including the molecule-of-interest attached thereto.

15 In nature there is an enormous variety of enzymes that catalyze reactions, some of which have industrial use. These include oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Immobilization of enzymes on solid substrates sometimes offers advantages over the use of a free enzyme. For example, immobilization can stabilize enzymes, enable better control of enzymatic reactions,
20 allow the reuse of the enzyme and prevent enzyme loss with time. The material bearing the immobilized enzyme has a significant role in evoking these advantages both from architectural and chemical points of view.

 Nanofibers and polymeric nanofibers in particular can be produced by an electrospinning process (Reneker DH., et al., 2006; Ramakrishna S., et al., 2005; Li D.,
25 et al., 2004; PCT WO 2006/106506 to the present inventors). Electrospun polymeric nanofibers have been widely used in biological applications such as scaffolds, carriers for biologically active molecules like proteins and enzymes (Xie J., et al., 2003; Zhang YZ., et al., 2006; Jiang H., et al., 2006; and Patel AC., et al., 2006) and encapsulation of viruses and bacteria (Salalha W., et al., 2006).

30 Several approaches can be used to entrap or attach enzymes to electrospun fibers. One approach is to immobilize the enzyme on the outer surface of the nanofibers by either covalently attaching the desired enzyme to the functional groups of the polymer

surface (Ye P., et al., 2006; Jia H., et al., 2002; Kim TG., et al., 2006) or physically absorbing the enzyme to the surface (Huang XJ., et al., 2006). The second approach, which results in encapsulation of enzymes, is based on mixing the enzyme with the polymer solution prior to the electrospinning process (Xie J. and Hsieh Y-L, 2003).
5 However, encapsulation is often associated with leaching of the enzymes, e.g., via fiber dissolution and burst releases (Zhang YZ., et al., 2006), especially, when the host polymer is a water soluble polymer such as poly(vinyl alcohol) (PVA) or dextran. To prevent immediate dissolution of the fibers in a physiological environment (e.g., blood) and the subsequent enzyme leaching, the electrospun fibers can be crosslinked by
10 chemical or physical agents such as glutaraldehyde or UV irradiation. Alternatively, Zeng J, et al. (2005) suggested that PVA fibers can be coated with water insoluble polymers using a chemical vapor deposition (CVD). However, the organic solvents of the water insoluble polymers are harmful to biological material and can lead to loss of enzymatic activity. To overcome this problem, Herricks et al. (2005) suggested to use
15 surfactant-stabilized enzymes in an organic solution of polystyrene (PS) as a spinning solution. In this way the electrospun nanofibers are insoluble in water and the enzymatic activity is retained due to surfactant stabilization (Herricks TE., et al., 2005).

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
20 two co-axial capillaries spinneret. 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 the present inventors (which is fully incorporated herein by reference) discloses methods of producing electrospun
25 microtubes (*i.e.*, hollow fibers) which can be further filled with liquids and be used as microfluidics.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is
30 provided a method of attaching a molecule-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
5 wherein the second polymeric solution comprises the molecule-of-interest, thereby attaching the molecule-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 molecule-of-interest attached to the microtube.

10 According to an aspect of some embodiments of the present invention there is provided a method of processing a substrate-of-interest, comprising contacting the substrate-of-interest with the microtube of the invention, wherein the molecule-of-interest is capable of processing the substrate, thereby processing the substrate-of-interest.

15 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 the invention, wherein the member of the affinity pair is selected capable of binding the molecule, thereby depleting the molecule from the solution.

20 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 the invention under conditions which allow binding of the molecule to the microtube via the member of the affinity pair which is selected capable of binding the molecule, and; (b) eluting the molecule from the microtube;
25 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 the invention, wherein the member of the affinity pair is selected capable of binding the molecule, and; (b) detecting binding of the
30 molecule by the member of the affinity pair; thereby detecting the presence of a molecule in the sample.

According to an aspect of some embodiments of the present invention there is provided a method of releasing a molecule-of-interest to cells of a subject in need thereof, comprising implanting in the subject the microtube of the invention, to thereby release the molecule-of-interest to cells of the subject.

5 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 solution.

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

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

 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),
15 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,
20 polyvinyl carbazole, polystyrene, poly(vinyl phenol), polyhydroxyacid, poly(caprolactone), polyanhydride, polyhydroxyalkanoate, polyurethane, collagen, albumin, alginate, chitosan, starch, 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),
25 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.

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

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

According to some embodiments of the invention, a 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.

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

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

10 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.

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

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

According to some embodiments of the invention, the molecule-of-interest is attached to the coat over the internal surface of the shell.

According to some embodiments of the invention, the molecule-of-interest is attached to the shell of the microtube.

30 According to some embodiments of the invention, the molecule-of-interest comprises a polypeptide, a polynucleotide, a carbohydrate, a small molecule, or any combination thereof.

According to some embodiments of the invention, the molecule-of-interest comprises a member of an affinity pair.

According to some embodiments of the invention, the polypeptide is an enzyme.

According to some embodiments of the invention, the enzyme is alkaline
5 phosphatase (SEQ ID NO:1 or 8) or beta-galactosidase (SEQ ID NO:2 or 9).

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

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

According to some embodiments of the invention, the shell prevents diffusion of
10 the molecule-of-interest therethrough.

According to some embodiments of the invention, the substrate-of-interest comprises incorporating the substrate-of-interest in a synthesis reaction catalyzed by the molecule-of-interest.

According to some embodiments of the invention, the substrate-of-interest
15 comprises incorporating the substrate-of-interest in a catabolism reaction catalyzed by the molecule-of-interest.

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

According to some embodiments of the invention, the solution comprises blood.

20 According to some embodiments of the invention, the affinity pair is selected from the group consisting of an enzyme and a substrate, a hormone and a receptor, an antibody and an antigen, a polypeptide and a polynucleotide, a polynucleotide and a cognate polynucleotide, a polypeptide and a metal ion, a polypeptide and a carbohydrate.

According to some embodiments of the invention, a therapeutically effective
25 amount of the molecule-of-interest is capable of treating a pathology in the subject.

According to some embodiments of the invention, the molecule-of-interest comprises a polypeptide, and whereas a therapeutically effective amount of the polypeptide is capable of treating a pathology in the subject.

According to some embodiments of the invention, the pathology is selected from
30 the group consisting of a metabolic disorder, an endocrine disease, an autoimmune disease, and cancer.

According to some embodiments of the invention, the polypeptide is selected from the group consisting of insulin (SEQ ID NO:6), phenylalanine hydroxylase (PAH) (SEQ ID NO:3), dystrophin (SEQ ID NO:4), beta-glucosidase (GBA) (SEQ ID NO:5), and ceruloplasmin ferroxidase (CP) (SEQ ID NO:7).

5 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 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
10 specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example
15 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 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.

20 In the drawings:

FIGs. 1A-D are images depicting high resolution scanning electron microscope (HRSEM) micrographs of (a) Type 1 fibers with alkaline phosphatase (AP); (b) Type 2 fibers with AP; (c) Type 1 fibers with beta-galactosidase (β -GAL); and (d) Type 2 fibers with β -GAL;

25 FIGs. 2A-C are graphs (Figures 2a-b) and a picture (Figure 2C) depicting the progress of the AP reactions with time for enzymes attached to (e.g., encapsulated within) the electrospun fibers and the free enzyme in the solution forming the coat over the internal surface of the shell (also referred to herein as a core solution) prior to the electrospinning. Figure 2A – the progress of the alkaline phosphatase reaction over
30 2500 minutes; Figure 2B – inset, the progress of the reaction through over the first 300 minutes; Note that the enzymatic reaction of the enzyme encapsulated within type 2 electrospun microtubes is faster than that of enzyme encapsulated within type 1

electrospun microtubes. Figure 2C – a photograph of a piece of mat (Type 1) immersed in the assay solution. The presence of the yellow reaction product, p-nitrophenol, is apparent; the reaction substrate was para-nitrophenyl phosphate;

FIG. 3 is a histogram depicting the relative activity of AP enzyme for different types of fibers (type 1 and type 2) and in dwelling buffers. Mat = the electrospun fibers (microtubes) with the attached (encapsulated) enzymes; rinsing buffer = the buffer used to only rinse the fibers, without any additional incubation time; 24 hrs. buffer = the buffer following incubation of the fibers therein for 24 hours; 72 hrs. buffer = the buffer following incubation of the fibers therein for 72 hours; core solution = the enzyme in the core solution prior to the electrospinning process.

FIGs. 4A-B are graphs depicting the progress of the β -GAL reactions with time for the two types of electrospun fibers and the free enzyme in the core solution. The substrate was ortho-nitrophenyl galactoside. Figure 4A – the progress of the β -GAL reactions over 5000 minutes as measured by the amount of ortho-nitrophenol generated; Figure 4B – inset of the graph of Figure 4a, the progress of the β -GAL reactions over the first 50 minutes;

FIG. 5 is a graph depicting the β -GAL reaction versus time for the mat and buffers for Type 2 fibers;

FIG. 6 is a histogram depicting the relative activity of the β -GAL and AP for different types of fibers;

FIGs. 7A-B are fluorescence microscope micrographs depicting Type 1 fibers with AP (Figure 7A) and β -GAL (Figure 7B). Size bars: 100 μ m (Figure 7A) and 50 μ m (Figure 7B).

FIG. 8 is a schematic illustration depicting the desorption process of the molecule-of-interest from the microtube of the invention. The molecule-of-interest (e.g., a protein, an enzyme) is attached to the coat over the internal surface of the shell. Following contacting the microtube with a solution, the solution enters the microtube via the pores (an exemplary pore is marked by arrow No. 3) by a capillary rise (see arrow No. 1) and gradually wets and fills the microtube inner volume. The desorption of the molecule-of-interest from the internal surface of the microtube shell (which

depends mainly on the rate of the release of the molecule-of-interest from the polymer) is shown by arrow No. 2.

FIG. 9 depicts a multi-step enzymatic reaction performed using encapsulated molecules-of-interest (enzymes 1-4). Enzyme 1 (enz1) catalyzes the conversion of compound A to B; Enzyme 2 (enz2) catalyzes the conversion of compound B to C; Enzyme 3 (enz3) catalyzes the conversion of compound C to D; Enzyme 4 (enz4) catalyzes the conversion of compound D to E.

FIG. 10 is a schematic presentation depicting Atrazine degradation by the isolated *Pseudomonas* ADP enzymes: AtzA (atrazine chlorohydrolase, e.g., GenBank Accession No. NP_862474), AtzB (hydroxyatrazine hydrolase, e.g., GenBank Accession No. NP_862481), AtzC (N-isopropylammelide isopropylamino hydrolase, e.g., GenBank Accession No. NP_862508), AtzD (cyanuric acid amidohydrolase, e.g., GenBank Accession No. NP_862537), AtzE (biuret hydrolase, e.g., GenBank Accession No. NP_862538) and AtzF (allophanate hydrolase, e.g., GenBank Accession No. AAK50333) which are attached to the microtube of the invention.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to methods of attaching a molecule-of-interest to a microtube and, more particularly, but not exclusively, to electrospun microtubes which include a molecule-of-interest attached thereto which can be used in various therapeutic, diagnostic, purification and synthesis 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 invention to practice, the present inventors have uncovered that a molecule-of-interest can be attached to electrospun microtubes. Thus, as is shown in Figures 1a-b and described in Example 1 of the Examples section which follows, two types of electrospun microtubes containing active molecules-of-interest (e.g., enzymes such as alkaline phosphatase or beta-galactosidase) were formed: Type 1 microtubes which exhibit a non-porous shell, and Type 2 microtubes which exhibit a porous shell

due to the presence of PEG in the polymeric solution forming the shell. The enzymatic activity contained within the microtubes was at the same order of magnitude as that of the polymeric solution prior to the electrospinning process (Figures 2a-b, 3, 4a-b and 6; Examples 1-3 of the Examples section which follows) indicating that surprisingly the process of production did not compromise the functionality of the delicate protein material incorporated into the tube. In addition, as is shown in Figure 3 and described in Example 2 of the Examples section which follows, both the porous and non-porous microtubes were capable of releasing enzymes attached thereto. Moreover, during the electrospinning process, some of the alkaline phosphatase enzyme migrated to the outer surface of the microtube shell and was released therefrom into the aqueous environment (Figure 3, Example 2), while the β -GAL enzyme remained within the internal surface of the shell (Figure 5, Example 3). In addition, as is further described in Example 3 of the Examples section which follows, the activity of the enzymes attached to the internal surface of the shell was increased in the presence of a porous shell which enabled the passage of substrates therethrough (Figures 4a-b). These results support the use of the microtubes of the invention as micro-reactors (e.g., bioreactors) for various synthesis, hydrolysis, isolation and purification reactions.

According to an aspect of the invention there is provided a method of attaching a molecule-of-interest to a microtube. The method is effected by 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 molecule-of-interest, thereby attaching the molecule-of-interest to the microtube.

As used herein the term "microtube" refers to a hollow tube having an inner diameter of e.g., about 200 nm to about 50 μ m and an outer diameter of e.g., about 0.5 μ m to about 100 μ m.

According to some embodiments of the invention the thickness of the microtube shell 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 internal diameter of the microtube shell can vary from a few nanometers to several micrometers, such as from
5 about 50 nm to about 50 μm , e.g., from about 100 nm to about 20 μm , from about 200 nm to about 10 μm , from about 500 nm to about 5 μm , from about 1 μm to about 5 μm , e.g., about 3 μm .

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
10 about 1-20 cm, e.g., from about 5-10 cm.

As used herein the term "attaching" refers to the binding of the molecule-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 immobilized molecule-of-interest
15 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) than the microtube's diameter (D). Accordingly, a molecule-of-interest which is attached to such a microtube is referred to as being entrapped or
20 encapsulated within the microtube.

According to some embodiments of the invention, covalent attachment of the molecule-of-interest 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
25 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
30 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.

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 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).

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 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 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 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.

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 of microtubes generated by electrospinning using different flow rates 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±0.1	8.5	0.43
	Second polymeric	0.5				

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
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 used was PCL 80 K; PEO used was PEO 600 K. The temperature during electrospinning was 22-26 °C. The relative humidity during electrospinning 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 (R) and the shell thickness (d) were measured in microns (μm). The voltage was measured in kilo volt (kV), and the electrostatic field was measured in kV per centimeter (cm). 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 derivatives thereof or, starches, poly(amino acids), poly(propylene fumarate), gelatin, alginate, pectin, fibrin, oxidized
5 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.
10 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
15 (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.

20 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. It will be appreciated that a biocompatible polymer can also be a biodegradable polymer.

25 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),
30 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 the 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 of the invention 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(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 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),
 5 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
 10 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 which are known in the art as capable of wetting other polymeric surfaces (for forming the shell) can be used. Following is a non-
 15 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
 20 ***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 (ε-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)
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)

<i>First polymeric solution forming the shell</i>	<i>Second polymeric solution capable of wetting the internal surface of the shell</i>
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.

- 5 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
- 10 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 CHCl_3 and DMF (90:10 by weight) as the first polymeric solution and 4 % (w/w) PEO in ethanol: H_2O (26:74 by weight) as a second polymeric solution.

5 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
10 water can wet a surface made of a PEO polymer. It will be appreciated that the wettability of the coat polymer covering the internal surface of the shell can be controlled (e.g., improved) for example by attaching functional groups such as hydroxyl groups (OH) which increase the hydrophilicity of the coat by a plasma treatment [see Thurston RM, Clay JD, Schulte MD, Effect of atmospheric plasma treatment on
15 polymer surface energy and adhesion, Journal of Plastic Film & Sheeting 23 (1): 63-78 JAN 2007;which is incorporated within by reference].

As is further discussed hereinabove and in the Examples section which follows, for certain applications the microtube shell may comprise pores, thus creating a "breathing" tube. Methods of forming "breathing" microtube (*i.e.*, microtubes with
20 pores in the shell thereof) are described in PCT/IB2007/054001 to the present inventors, 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
25 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
30 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
5 (>) than 150 nm in diameter, the first polymeric solution may include about 4 % PEG MW 35 kDa. Similarly, to generate pores smaller (<) than 150 nm in diameter, the first polymeric solution may include about 2 % PEG MW 6 kDa.

The microtube shell of the invention can be designed such that it enables the passage of certain molecules (e.g., a substrate of an enzyme) while preventing the
10 passage of other molecules (e.g., a certain enzyme), depending on the geometry (pore size) and/or the electrical charge of the molecules with respect to the geometry (length and radius), surface energy, electrical charge of the nanopore(s) of the shell, and the viscosity and surface tension of the liquid containing the molecules (e.g., the substrate of the enzyme). In addition, the porosity and pore size of the shell can control the
15 release of the molecule-of-interest which is attached to the microtube. For example, a higher porosity and/or pore size can result in increased rate of release of the molecule-of-interest.

Alternatively, the microtube shell can be made such that it prevents diffusion or any passage of the molecule-of-interest therethrough (*i.e.*, substantially devoid of pores,
20 or with pores smaller than the molecule-of-interest).

As mentioned, the second polymeric solution comprises the molecule-of-interest. Such a molecule (or molecules) can be any naturally occurring or synthetic molecule such as a polypeptide, a polynucleotide, a carbohydrate or a polysaccharide, a lipid, a drug molecule, a small molecule (e.g., a nucleotide base, an amino acid, a
25 nucleotide, an antibiotic, a vitamin or a molecule which is smaller than 0.15 kDa), or any combination thereof. The molecule-of-interest can be produced by recombinant DNA technology or by known synthesis methods such as solid phase.

According to some embodiments of the invention, the molecule-of-interest comprises a polypeptide such as an enzyme. Such polypeptides (e.g., enzymes) can be
30 naturally occurring (e.g., mammals such as primates, rodents and Homo sapiens, plants, fungi, protozoa, bacteria and viruses) or synthetic (e.g., derived from in vitro evolution) and can be selected according to the desired application.

The following non-limiting list of enzymes can be attached to the microtube of the invention: DNA polymerase (EC 2.7.7.7), DNase (EC 3.1.11.4), RNA polymerase (EC 2.7.7.6), DNA ligase (EC 6.5.1.1), RNA ligase (EC 6.5.1.3), alcohol dehydrogenase (EC 1.1.1.1), homoserine dehydrogenase (EC 1.1.1.3), acetoin dehydrogenase (EC 1.1.1.5), glycerol dehydrogenase (EC 1.1.1.6), L-xylulose reductase (EC 1.1.1.10), L-arabinitol 2-dehydrogenase (EC 1.1.1.13), L-iditol 2-dehydrogenase (EC 1.1.1.14), mannitol-1-phosphate 5-dehydrogenase (EC 1.1.1.17), mannitol 2-dehydrogenase (EC 1.1.1.138), glucose oxidase (EC 1.1.3.4), L-sorbose oxidase (EC 1.1.3.11), lactate-malate transhydrogenase (EC 1.1.99.7), formaldehyde dehydrogenase (EC 1.2.1.1), aryl-aldehyde dehydrogenase (EC 1.2.1.29), aldehyde oxidase (EC 1.2.3.1), pyruvate synthase (EC 1.2.7.1), cortisone α -reductase (EC 1.3.1.4), lathosterol oxidase (EC 1.3.3.2), D-proline reductase (EC 1.4.4.1), dihydrofolate reductase (EC 1.5.1.3), methylenetetrahydrofolate reductase (NADPH) (EC 1.5.1.20), cystine reductase (NADH) (EC 1.6.1.4), cob(II)alamin reductase (EC 1.6.99.9), sulfite reductase (EC 1.8.1.2), cytochrome-c oxidase (EC 1.9.3.1), NADH peroxidase (EC 1.11.1.1), homogentistate 1,2-dioxygenase (EC 1.13.11.5), *Photinus*-luciferin 4-monooxygenase (1.13.12.7), anthranilate 3-monooxygenase (EC 1.14.13.35), steroid 9 α -monooxygenase (EC 1.14.99.25), mercury(II) reductase (EC 1.16.1.1), nicotinamide *N*-methyltransferase (EC 2.1.1.1), thymidylate synthase (EC 2.1.1.45), site-specific DNA-methyltransferase (adenine-specific) (EC 2.1.1.72), tryptophan 2-C-methyltransferase (EC 2.1.1.106), glycine formiminotransferase (EC 2.1.2.4), aspartate carbamoyltransferase (EC 2.1.3.2), transaldolase (EC 2.2.1.2), arylamine *N*-acetyltransferase (EC 2.3.1.5), arginine *N*-succinyltransferase (EC 2.3.1.109), phosphorylase (EC 2.4.1.1), glycosaminoglycan galactosyltransferase (EC 2.4.1.74), thymidine phosphorylase (EC 2.4.2.4), β -galactoside α -2,6-sialyltransferase (EC 2.4.99.1), galactose-6-sulfurylase (EC 2.5.1.5), aspartate transaminase (2.6.1.1), hexokinase (EC 2.7.1.1), choline kinase (EC 2.7.1.32), acetate kinase (EC 2.7.2.1), creatine kinase (EC 2.7.3.2), adenylate kinase (EC 2.7.4.3), nucleotide pyrophosphokinase (EC 2.7.6.4), sulfate adenyltransferase (ADP) (EC 2.7.7.5), aryl sulfotransferase (EC 2.8.2.1), carboxylesterase (3.1.1.1), acetyl-CoA hydrolase (EC 3.1.2.1), alkaline phosphatase (3.1.3.1), phosphodiesterase I (EC 3.1.4.1), dGTPase (EC 3.1.5.1), steryl-sulfatase (EC 3.1.6.2), exodeoxyribonuclease I (EC 3.1.11.1),

ribonuclease T1 (EC 3.1.27.3), α -amylase (EC 3.2.1.1), purine nucleosidase (EC 3.2.2.1), epoxide hydrolase (EC 3.3.2.3), lysyl aminopeptidase (EC 3.4.11.15), carboxypeptidase A2 (EC 3.4.17.15), trypsin (EC 3.4.21.4), glutaminase (EC 3.5.1.2), barbiturase (EC 3.5.2.1), ATP deaminase (EC 3.5.4.18), inorganic pyrophosphatase (EC 3.6.1.1), oxaloacetase (EC 3.7.1.1), oxalate decarboxylase (EC 4.1.1.2), mandelonitrile lyase (EC 4.1.2.10), isocitrate lyase (4.1.3.1), fumarate hydratase (EC 4.2.1.2), pectate lyase (EC 4.2.2.2), histidine ammonia-lyase (EC 4.3.1.3), cyanate lyase (4.3.99.1), cysteine lyase (EC 4.4.1.10), DDT-dehydrochlorinase (EC 4.5.1.1), adenylate cyclase (EC 4.6.1.1), alanine racemase (5.1.1.1), tartrate epimerase (EC 5.1.2.5), retinal isomerase (EC 5.2.1.3), L-rhamnose isomerase (EC 5.3.1.14), prostaglandin-D synthase (EC 5.3.99.2), phosphoglucomutase (EC 5.4.2.2), lanosterol synthase (EC 5.4.99.7), DNA topoisomerase (EC 5.99.1.2), tyrosine-tRNA ligase (EC 6.1.1.1), acetate-CoA ligase (EC 6.2.1.1), acetylcholinesterase (EC 3.1.1.7), butyrylcholinesterase (EC 3.1.1.8) and glutathione synthase (EC 6.3.2.3).

According to an embodiment of the invention, the enzyme which is attached to the microtube is alkaline phosphatase (e.g., SEQ ID NO:1 or 8; EC 3.1.3.1) or beta-galactosidase (e.g., SEQ ID NO:2 or 9; EC 3.2.1.23).

The term "polynucleotide" as used herein refers to a single stranded or double stranded oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes polynucleotides composed of naturally-occurring bases, sugars and covalent internucleoside linkages (e.g., backbone) as well as polynucleotides having non-naturally-occurring portions which function similarly to respective naturally-occurring portions.

The polynucleotide which is attached to the microtube of the invention can be generated according to any oligonucleotide synthesis method known in the art such as enzymatic synthesis, liquid phase or solid phase synthesis (using a commercially available equipment from, for example, Applied Biosystems). Equipment and reagents for executing solid-phase synthesis are commercially available from, for example, Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the capabilities of one skilled in the art and can be accomplished via established methodologies as detailed in, 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) and "Oligonucleotide Synthesis" Gait, M. J., ed. (1984) utilizing solid phase chemistry, e.g. cyanoethyl phosphoramidite followed by deprotection, desalting and purification by for example, an automated trityl-on method or HPLC. Liquid phase synthesis of oligonucleotides can be performed using methods known in the art (see for example, Bonora GM, et al., 1998, Biol. Proced. Online. 1: 59-69; Padiya KJ and Salunkhe MM., 2000, Bioorg. Med. Chem. 8: 337-42). It will be appreciated that for the preparation of multiple labeled polynucleotides, a large scale oligonucleotide synthesis can be utilized essentially as described elsewhere (Anderson NG. et al., Appl Biochem Biotechnol. 1995 Jul-Sep;54(1-3):19-42; Rahmann S., Proc IEEE Comput Soc Bioinform Conf. 2002;1:54-63).

Additionally or alternatively, the polynucleotide which is attached to the microtube of the invention can be generated by recombinant DNA techniques using any known DNA replication or transcription system (e.g., using bacterial cells, eukaryotic cells).

As mentioned, the molecule-of-interest can be a drug molecule. Such a drug can be any synthetic, chemical or biological molecule.

Non-limiting examples of biological drug molecules include antisense oligonucleotides, Ribozymes, DNazymes, siRNA, receptor agonists, antagonists, hormones, growth factors and antibodies. Non-limiting examples of which chemical drug molecules include chemotherapy agents, Paclitaxel (Taxol®), radiation seed particles (e.g., see Hypertext Transfer Protocol://World Wide Web (dot) oncura (dot) com), as well as natural or synthetic vitamins.

The molecule-of-interest which is attached to the microtube of the invention can be labeled. Such a label can be an intrinsic property of the molecule-of-interest (e.g., as in the case of green fluorescent protein) or can be a label which is attached to the molecule-of-interest using known methods. For example, the label can be a fluorescent labeling in which a fluorophore (*i.e.*, an entity which can be excited by light to emit fluorescence) or a radio-isotope is conjugated via a linker or a chemical bond to the molecule-of-interest. Alternatively, the molecule-of-interest can be indirectly labeled

via a covalently conjugated enzyme (e.g., horse radish peroxidase) and a covalently conjugated substrate (e.g., o-phenylenediamine) which upon interaction therebetween yield a colorimetric or fluorescent color.

The molecule-of-interest can also comprise a member of an affinity pair, which is capable of reversibly or non-reversibly binding with high affinity (e.g., less than 10^{-7} M, e.g., less than 10^{-8} M, less than 10^{-9} , less than 10^{-10} M) to a specific molecule. For example, the affinity pair can be an enzyme-substrate pair, a polypeptide-polypeptide pair (e.g., a hormone and receptor, a ligand and receptor, an antibody and an antigen, two chains of a multimeric protein), a polypeptide-small molecule pair (e.g., avidin or streptavidin with biotin, enzyme-substrate), a polynucleotide and its cognate polynucleotide such as two polynucleotides forming a double strand (e.g., DNA-DNA, DNA-RNA, RNA-DNA), a polypeptide-polynucleotide pair (e.g., a complex formed of a polypeptide and a DNA or RNA e.g., aptamer), a polypeptide-metal pair (e.g., a protein chelator and a metal ion), a polypeptide and a carbohydrate (leptin-carbohydrate), and the like.

The molecule-of-interest, which is comprised within the second polymeric solution, can be attached to the coat over the internal surface of the shell. For example, as shown in Figure 5 and described in Example 3 of the Examples section which follows, most of the β -GAL enzymatic activity was detected inside the microtube, demonstrating the attachment of the enzyme to the coat over the internal surface of the shell.

During the electrospinning process some molecules-of-interest which are comprised within the second polymeric solution may migrate to the outer surface of the shell (i.e., mixed with the first polymeric solution) depending on their charge state, size and geometry. For example, as shown in Figure 3 and described in Example 2 of the Examples section which follows, some of the alkaline phosphatase activity was detected in the rinsing buffer of the microtube.

According to some embodiments of the invention attachment of the molecule-of-interest is performed following microtube formation. For example, the microtube can be soaked with a solution containing the molecule-of-interest. The molecule-of-interest can diffuse through the shell pores and enter the inner lumen of the microtube.

In addition, the microtube can be covalently attached to the molecule-of-interest (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
5 surface of the shell and a molecule-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
10 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
15 dimensions of the microtube which are unique to electrospinning.

The microtube of the invention can be 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
20 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
25 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 fibers (A. Theron, E. Zussman, A.L. Yarin, "Electrostatic field-assisted alignment of
30 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 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 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 spun dry microtubes) or as a wetted mat(s) (e.g., following immersing or filling the microtube with a liquid).

The microtube of the invention which is attached to the molecule-of-interest may be configured as or in a microfluidics device. "Lab-on-a-chip" is described in a series of review articles [see for 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 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 in or surrounds the microtube enables the desorption (detachment) of the molecule-of-interest 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 molecule-of-interest and a

substrate. According to some embodiments of the invention the desorption process enables the flow and/or the release of the molecule-of-interest within and/or from the microtube.

According to some embodiments of the invention, the molecule-of-interest which is attached to the microtube of the invention remains active, *i.e.*, maintains the activity, or at least a portion thereof, which it possessed prior to the attachment (e.g., of the same molecule-of-interest before electrospinning, or when not-attached to the microtube). The term "activity" as used herein refers to any of a catalytic activity, kinetics, and/or affinity to a substrate, a ligand or an affinity member of the molecule. Such an activity can be any biological activity such as catalysis, binding (with a specific affinity), hybridization, chelation, degradation, synthesis, catabolism, hydrolysis, polymerization, transcription, and the like.

As used herein the phrase "at least a portion of the activity" refers to at least 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 molecule-of-interest possessed prior to the attachment to the microtube.

For example, as mentioned before and described in the Examples section which follows, the enzymes contained within the microtubes preserved the specific activity to their substrates at a kinetic which is comparable (*i.e.*, within the same order of magnitude) to that of the enzyme in the polymeric solution prior to electrospinning.

The microtube of the invention which is attached to an active molecule-of-interest can be used in various applications which require the attachment of active molecules (e.g., enzymes, DNA, RNA) 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 molecule-of-interest. The combination of molecules can be selected according to the intended use. For example, several molecules (e.g., enzymes) which are involved in complex reactions (e.g., processing of a substrate or a mixture of substrates) can be used.

Thus, 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 molecule-of-interest 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 molecule-of-interest which is attached to the microtube on its cognate substrate.

5 According to some embodiments of the invention, such a process can concomitantly incorporate of the substrate-of-interest in a synthesis reaction catalyzed by the molecule-of-interest.

For example, the microtube of some embodiments the invention can be used as a micro-reactor (e.g., bioreactor) for chemical transition reactions requiring high
10 concentrations of several enzymes. As described in Example 4 of the Examples section which follows and schematically illustrated in Figure 9, the microtube of the invention can be attached to certain molecules (enzymes in this case), which together catalyze a multi-step synthesis reaction (e.g., cascade) which converts an initial substrate (e.g., compound A) to an end-product (e.g., compound E). As mentioned, the selective shell
15 of the microtube can be designed such that it prevents the leakage (escape by diffusion) of the intermediate compounds (e.g., compounds B, C and D) therethrough and thus enables sufficiently high concentrations of such compounds as needed for the synthesis of the end product. The local concentration of the intermediate molecules formed from the initial substrate (entrapped at the time of spinning or externally added after
20 electrospinning to the formed microtube) are about 2-10 orders of magnitude greater than the concentrations formed in an open system. Thus, the microtube of some embodiments of the invention exhibits a great kinetic advantage in multi-step reactions as compared to an open system. Microtubes are in this way similar to living cells which function on the same principle.

25 For example, to synthesize an indole-glycerol phosphate, an intermediate compound in tryptophan synthesis within cells, a microtube of some embodiments of the invention can be attached to the enzymes anthranilate-phosphoribosyl transferase (EC 2.4.2.18), phosphoribosylanthranilate isomerase (EC 5.3.1.24) and indole-3-glycerol-phosphate synthase (EC 4.1.1.18), and the reaction commences when anthranilate and
30 phosphoribosyl-pyrophosphate interact with the attached enzymes. The substrates (anthranilate and phosphoribosyl-pyrophosphate) can be either added externally to the reaction medium (within which the microtube is placed) or can be attached to the

microtube by mixing them within the second polymeric solution. When the substrates are supplied externally, pores of about 2-20 nm in diameter should exist in the shell to allow diffusion of anthranilate and phosphoribosyl-pyrophosphate therethrough.

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 molecule-of-interest.

A catabolism reaction can be the degradation (e.g., by hydrolysis) of a toxic molecule for the purpose of detoxification (e.g., detoxifying water) or decomposition of an unwanted molecule. Examples include, but are not limited to, the removal of the chlorine entity from atrazine (see Figure 10) and the degradation of cyanide resulting from silver mining.

According to an aspect of the invention, there is provided a method of depleting a molecule from a solution. The method is effected by contacting the solution with the microtube of the invention, wherein the member of the affinity pair (which is attached to the microtube) is selected capable of binding the molecule (which is to be removed), thereby depleting the molecule from the solution.

According to an embodiment of the invention, the method further comprising collecting the solution following the contacting.

As used herein the phrase "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 solution.

According to some embodiments of the invention, the depletion (removal) of the molecule from the solution is effected within a short time period, such as within minutes (e.g., 1-30 minutes), hours (e.g., 1-10 hours) or several days (e.g., 1-5 days).

As used herein the phrase "contacting" refers to enabling the interaction between the molecule and the member of the affinity pair, which is attached to the microtube, for a time period which is sufficient for depleting the molecule from the solution. Such a contact can take place while the 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 member of the affinity pair can take place by incubating the microtube in the solution (e.g., by placing the microtube in a container including the solution).

The solution can be any water-based solution which includes inorganic or organic molecules, such as a biological sample or a sample from a non-living source such as stream or ocean waters. As used herein the phrase "biological sample" refers to any sample derived from a living organism such as plant, bacteria or mammals, and can include cells or alternatively be cell-free (i.e., include only a biological fluid). For example, a biological sample of an individual can include body fluids such as blood or components thereof (e.g., white blood cells, red blood cells, coagulation factors, leukocytes, neutrophils, serum, plasma), cerebrospinal fluid, urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk, amniotic fluid and chorionic villi, a tissue biopsy, a tissue section, a malignant tissue, and the like. The sample can be derived from the individual and be further tested *in vitro* or *ex vivo*, or alternatively, can be not physically removed from the subject (e.g., for *in situ* detection and/or diagnosis).

According to some embodiments of the invention, the solution is an aqueous solution such as a drinking water, a groundwater and/or an industrial waste water. According 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).

For example, to remove a certain metal ion (e.g., copper, gold, nickel, zinc, lead, mercury, cadmium, silver, iron, manganese, palladium, and platinum) from water, the microtube of the invention can be attached to a water soluble ethylene dichloride ammonia polymer, which contains dithiocarbamate salt groups and is capable of chelating the metal ion (US Pat. No. 5,346,627). Thus, by contacting the water with the microtube the ethylene dichloride ammonia polymer binds to the metal ion and removes it from the water. Water collected after being in contact with the microtube is substantially devoid of the metal ion. Alternatively, these metal ions may be removed by attaching a protein chelator of such metal ions to the microtube.

Alternatively, to remove a ligand (e.g., a hormone, a substrate, a co-factor or a vitamin such as biotin) from a solution containing a biological sample, the microtube can be attached to a polypeptide which is member of an affinity pair such as an enzyme, a hormone or streptavidin, and following contacting the solution with the microtube, the ligand remains attached to the microtube while the solution is substantially devoid of the

ligand (e.g., includes less than 0.5 %, e.g., less than 0.1 %, e.g., less than 0.01 % of the ligand).

According to some embodiments of the invention, the molecule which is to be removed from the solution comprises an antigen and the member of the affinity pair
5 comprises the antibody capable of specifically binding the antigen.

For example, the microtube of the invention can be used to remove virus particles from a blood sample. Briefly, an anti-virus antibody (e.g., anti-HIV antibodies such as those described in Tullis, RH., et al., Therapeutic Apheresis and Dialysis, 6: 213-220) can be attached to the microtube and a blood sample containing the virus
10 particles (e.g., HIV particles) can be in contact with the microtube such that the virus particles bind to their respective antibodies and the collected blood sample (after being in contact with the microtube) is substantially devoid of the viral particles.

The member of the affinity pair which is attached to the microtube of the invention can be also used to isolate a molecule from a solution.

According to 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 the microtube of the invention under conditions which allow binding of the molecule to the microtube via the member of the affinity pair which is selected capable of binding the molecule, and (b) eluting the molecule from the microtube, thereby isolating the
15 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 member of the affinity pair that is attached to the microtube and eluting the bound molecule therefrom. As used herein the term "eluting" refers to dissociating the bound molecule from the
20 microtube. Those of skills 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 other member of the affinity pair.

As is further described in Example 5 of the Examples section which follows, the present inventors have envisaged the use of the microtube of the invention, which is
25 attached to a member of an affinity pair, 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 member of the affinity pair is conjugated to a solid support not

having a tubular structure, such as a flat support) due to the increased ratio between the size of the microtube surface (which attaches the member of the affinity pair) and the volume of the sample being in contact therewith.

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 member of the affinity pair is selected capable of binding the molecule, and; (b) detecting binding of the molecule by the member of the affinity pair, thereby detecting the presence of a molecule in the sample.

As used herein the phrase “detecting binding” refers to identifying a change in the concentration, conformation, spectrum or electrical charge of the molecule in the sample and/or the member of the affinity pair that is attached to the microtube following the binding therebetween. Identification of such binding can be performed using methods known in the art such as following the fluorescence or the color of the sample, radioactivity in the sample, the electrical conductivity of the sample and the like.

As mentioned hereinabove and described in Example 2 of the Examples section which follows, the microtube of the invention can release the molecule-of-interest attached thereto (a releasing apparatus).

The microtube of some embodiments 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 condition) which can be treated by the molecule that is attached to or flows through 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 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.

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 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.

For example, the microtube of the invention which is capable of depleting a molecule from a solution as described above, can be connected to a blood vessel of the subject. For example, the proximal end of the microtube (or of a plurality of microtubes) can be connected to a feeding blood vessel and the distal end of the microtube(s) can be connected to a receiving blood vessel. Such a configuration can be used, for example, for hemodialysis and depletion of a specific molecule (e.g., a virus particle such as HIV, hepatitis virus such as HCV) from the blood stream of the subject.

In addition, a microtube which is attached to a drug molecule can be implanted in a subject in need thereof to thereby release a therapeutically effective amount of the drug to cells of the subject.

- As used herein the phrase "therapeutically effective amount" means an amount of the molecule-of-interest (e.g., the drug, the active molecule) effective to prevent, alleviate or ameliorate symptoms of a pathology or prolong the survival of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

For example, in case the molecule-of-interest comprises a polynucleotide, such a polynucleotide can be used in gene therapy applications to either increase an expression level or activity of a desired polypeptide needed for treating the pathology, or to decrease or inhibit the expression level of a polynucleotide causing the pathology (e.g., antisense technology). Alternatively, the polynucleotide can be used to immunize the subject by inducing an immune response thereagainst.

Alternatively, in case the molecule-of-interest is a polypeptide, such a polypeptide can be used in a subject in need of polypeptide therapy, such as a subject having a decreased or no activity of the polypeptide (e.g., due to a genetic disease, auto-antibodies, pathogen infection, degeneration or a decrease in tissue functioning), as well as for other therapeutic applications such as immunization with the polypeptide.

Non-limiting examples of pathologies which require polypeptide therapy and can be treated using the microtube of the invention include, metabolic disorders such as phenylketonuria (PKU), Gaucher disease, muscular dystrophy [Duchenne (DMD) and

Becker (BMD) Muscular Dystrophies], Aceruloplasminemia (an iron metabolic disorder), endocrine diseases such as diabetes, autoimmune diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA), and psoriasis, and various cancers (e.g., lymphoma).

5 Following is a non-limiting list of polypeptides which can be attached to the microtube of the invention in order to treat pathologies requiring polypeptide therapy. Phenylalanine hydroxylase [(PAH); GenBank Accession Nos. NM_000277.1 (nucleic acid sequence) and NP_000268.1 (SEQ ID NO:3; amino acid sequence)] for treating phenylketonuria (PKU), dystrophin [(DMD); GenBank Accession Nos. NM_000109.2
10 (nucleic acid sequence) and NP_000100.2 (SEQ ID NO:4; amino acid sequence)] for treating Duchenne (DMD) and Becker (BMD) Muscular Dystrophies, beta-glucosidase [(GBA); GenBank Accession Nos. NM_001005741.1 (nucleic acid sequence) and NP_001005741.1 (amino acid sequence; SEQ ID NO:5)] for treating Gaucher disease, insulin [GenBank Accession Nos. NM_000207.1 (nucleic acid sequence) and
15 NP_000198.1 (amino acid sequence; SEQ ID NO:6)] for treating diabetes, and ceruloplasmin ferroxidase [(CP); GenBank Accession Nos. NM_000096.1 (nucleic acid sequence) and NP_000087.1 (SEQ ID NO:7; amino acid sequence)] for treating aceruloplasminemia, CD20 monoclonal antibodies for treating non-Hodgkin's lymphoma and autoimmune disease (Yazawa N, et al., 2005, Proc Natl Acad Sci USA.
20 102:15178-83) and T-cell receptor peptides for treating of multiple sclerosis (MS), rheumatoid arthritis (RA), and psoriasis (Vandenbark AA, et al., 2001, Neurochem Res. 26:713-30).

Targeted delivery of a drug molecule to a tissue-of-interest is desired in various pathologies, especially in cases where the effect of the drug is deleterious to non-
25 diseased tissues or when high concentrations of drug molecules are required to achieve a therapeutic effect on the diseased tissue (the tissue-of-interest). Thus, for example it is highly desired to have a targeted delivery of a chemotherapy agent or a radiation seed particle to the liver in case of hepatic cancer, or an angiogenic factor to coronary blood vessels, heart or carotid blood vessels in case of ischemia.

30 According to an embodiment of the invention, for targeted delivery of a drug molecule to a tissue-of-interest via the opening of the microtube (at the targeted tissue), the microtube of the invention is designed such that the electrospun shell is semi-

permeable (*i.e.*, prevents passage of the drug molecule but enables the penetration of water or a physiological solution therethrough) and the coat over the internal surface of the shell is attached to the drug molecule.

Such a microtube can be implanted in a subject such that the distal end of the microtube is implanted in or in close proximity to the tissue-of-interest. As used herein the term "proximity" refers to being in a cavity defined by the tissue, for example, if the tissue in which the drug is released is a blood vessel (artery or vein) the cavity is a lumen of such a blood vessel, or if the tissue in which the medication is released is a heart chamber, then the cavity is an atrium or a ventricle. It will be appreciated that the other end of the microtube can be also implanted in proximity to the tissue-of-interest. Alternatively, the proximal end of the microtube can be either sealed using *e.g.*, a laser beam to prevent delivery of the drug to undesired cells/tissues of the subject, or if needed, could be placed outside the body, or subcutaneously such that the microtube can be replenished with additional drug molecules using extra thin needles (*e.g.*, which can penetrate a 5 μm lumen of the microtube).

Once the microtube of some embodiments of the invention (*e.g.*, a microtube with a semi permeable shell and a drug molecule attached to the coat over the internal surface of the shell) is implanted in the subject it can be filled with a physiological fluid (*e.g.*, of the subject) which is capable of dissolving the water-soluble polymer of the coat over the internal surface of the shell to thereby release the drug molecule therefrom. The released drug molecule flows by capillary forces within the microtube until reaching the end of the open lumen, which is in proximity of the tissue-of-interest.

If needed, the microtube according to this embodiment of the invention, can be also replenished with additional drug molecules or other molecules which can increase the effect of the drug molecule released by the microtube. For example, if the drug molecule attached to the microtube is an angiogenic factor, a solution saturated with gasses (*e.g.*, oxygen) can be administered to the microtube (*e.g.*, after implantation in the subject) to thereby increase the anti-ischemic effect of the angiogenic factor.

Targeted delivery of a drug to a tissue-of-interest can be also effected using a microtube in which the shell enables diffusion of the drug molecule therethrough and accordingly, the drug molecule can be released through the shell pores and/or the distal opening of the microtube at the desired tissue.

The invention further envisages the use of the microtube of the invention, which include a molecule-of-interest attached thereto, for guiding cell growth *ex vivo* or *in vivo*. For example, neuronal cells can be placed near or in direct contact with the microtube which is attached to necessary growth factors and 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 and the 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.

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

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to". This term encompasses the terms "consisting of" and "consisting essentially of".

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

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 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 subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges 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 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.

An electrospinning apparatus can include a controller programmed with parameters as described herein or measuring output and automatically modifying. Controller can be hardware, software, firmware, with CPU, volatile memory, optional non-volatile memory.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

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.

30

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

Enzymes and 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. Alkaline phosphatase (AP) and beta-galactosidase (β -GAL) from *E. coli* were also purchased from Sigma-Aldrich. AP cleaves monophosphate esters and has a molecular weight of about 80,000 Da. β -GAL is a tetrameric enzyme of 465,396 Da consisting of four identical subunits each (Zabin I., et al., 1980) and catalyzes the hydrolysis of the terminal galactosidyl group of β -galactosides. Both enzymes were initially dissolved in water and then mixed with the core solution.

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 + 0.0733 mg/ml AP or 2.38 units/ml β -GAL; in ethanol: H_2O (26:74 by weight)
2	10 % PCL 80 K + 1 % PEG 6 K; in CHCl_3 :DMF (90:10 by weight)	4 % (w/w) PEO 600 K + 0.0733 mg/ml AP or 2.38 units/ml β -GAL; in ethanol: H_2O (26:74 by weight)

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).

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. 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.

Measurement of the Enzyme Activity - To measure enzyme activity, pieces of mat were weighed and dipped in assay solution according to Table 4, hereinbelow. At each time of sampling, the solution was mixed with a vortex mixer, and 1 ml of the assay mixture was transferred to spectrophotometer cuvette. The absorbance of the solution was measured in a Perkins-Elmer spectrophotometer at a wavelength of 410 nm. For both enzymatic reactions, the substrates are colorless but the products, para-nitrophenol for AP and ortho-nitrophenol for β -GAL, are yellow with an absorption maximum at 410 nm. After the absorbance was measured, the liquid was returned to the assay vessel. Units, activity and relative activity are defined as follow:

$$(1) \quad Unit = \frac{\Delta A}{\Delta t} \cdot 1000$$

$$(2) \quad Activity \text{ of the mat} = unit \cdot C$$

$$20 \quad \text{where} \quad C = \frac{\text{mass of total mat}}{\text{mass of piece}}$$

$$(3) \quad Re\ lative \ Activity \ (\%) = \frac{Activity \ of \ the \ mat}{Activity \ of \ the \ core \ solution} \cdot 100$$

Where: ΔA is the difference is the absorbance at difference times, Δt , difference between t_1 and t_2 (two time points) taken in the linear region of the reaction curve, and C is a normalization factor which takes into account the different weight of each piece.

For the fluorescence microscope imaging a drop of the assay solution was put directly on the microscope slide on which a few fibers had been deposited. The

fluorescent substrates were methylumbelliferyl-phosphate for alkaline phosphatase (AP) and methylumbelliferyl-galactoside for beta-galactosidase (β -GAL). These were used at the same concentrations as their nitro-phenyl analogs.

5

Table 4
Composition of the assays

Enzyme	Substrate	Buffer	H₂O	Products
AP	0.7 mg/ml p-nitrophenyl-phosphate (MW = 217 Da) 1 ml	TRIS-HCl buffer 1.5 ml	0.5 ml	p-nitrophenol + PO ₄
	4-methylumbelliferyl-phosphate: fluorescence microscopy			4-methylumbelliferone + PO ₄
β -GAL	4 mg/ml o-nitrophenyl- β -D-galactoside 0.2 ml (MW = 301 Da)	Z-buffer 0.7 ml	0.3 ml	o-nitrophenol + galactose
	4-methylumbelliferyl- β -D-galactoside: fluorescence microscopy			4-methylumbelliferone + galactose

Table 4.

10

EXAMPLE 1

ATTACHMENT OF ENZYMES TO ELECTROSPUN MICROTUBES

Experimental Results

Formation of micro-tubes - Two types of electrospun hollow fibers have been fabricated with the polymers listed in Table 3, hereinabove. The resultant fibers are hollow structures, namely micro-tubes, as previously described by the present inventors (Dror, Y et al., 2007 and PCT/IB2007/054001, which is fully incorporated herein by reference). The hollow nature of these structures and the different morphologies of the tube walls are demonstrated in the high resolution scanning electron microscope (HRSEM) micrographs presented in Figures 1a-d. Type 1 fibers are made with only PCL in the shell and exhibit a rough surface due to the rapid evaporation of the solvent (Figures 1a and c). However, this roughness doesn't affect the intact nature of the walls. As PEG is added to the shell solution (Type 2), the walls become increasingly porous (Figures 1b and d) and pores can be seen even in the interior surface of the tubes (Figure

1b). PEG and PCL are partially miscible due to favorable, but weak, intermolecular polar-interactions (Coleman MM, et al., 1991). During fiber solidification along with the evaporation of the solvents, the concentration of the components increases and phase separation takes place. However, since the PEG has a surfactant-like character it deposits an adherent film around the PCL domains resulting in the formation of pores rather than forming solid domains of PEG.

After electrospinning the tubes form a fibrous non-woven, aligned or non-aligned mat. When the mat material is placed in an aqueous environment, the coat over the internal surface of the shell dissolves and the enzymes are released (through desorption) and become active on their substrate(s). For a further description of the desorption process see Figure 8 and Example 8 hereinbelow. This arrangement of material allows for flow-through technologies without subjecting the enzyme to the external environment and without the need for chemical attachment and can prevent loss of the entrapped enzyme.

Enzymes attached to fibers maintain normal biological activity - The kinetics of the enzymatic reaction for alkaline phosphatase was measured as described in the experimental section and is presented in Figures 2a-b. The enzymatic reaction with the fibers was compared to the free enzyme in the core solution (before electrospinning) and normalized with respect to the weight of the analyzed pieces. The results strongly indicate that the enzyme attached in the fiber (e.g., encapsulated) maintains its biological activity after electrospinning and exhibits a reaction curve similar to the free enzyme. The curves are characteristic of enzymatic reactions when there is large excess of substrate. The colored product, p-nitrophenol, diffuses out of the fibers into the surrounding medium as shown in Figure 2c (Type 1). The reaction rates of the enzyme attached (e.g., contained within) the microtubes are slightly reduced in comparison to the free enzyme. Without being bound by any theory, it is possible that the reaction rates are reduced because of the following reasons: (1) the substrate has to diffuse into the fibers in order to reach the active site of the enzyme; and/or (2) the enzyme has to diffuse outside; (3) the product has to diffuse outside in order to be detected; (4) some enzyme activity was lost during the spinning process itself. The attainment of maximum reaction velocity occurs more rapidly with Type 2 fibers, undoubtedly due to their highly porous character. Upon closer examination of the initial kinetics, it can be seen that

Type 1 fibers, which do not contain PEG in the shell, exhibit a linear reaction rate that is much reduced (inset- Figure 2b, marked by dark arrows) than Type 2 fibers (which include PEG in the shell polymer, and consequently, pores in the microtube shell). Without being bound by any theory, the initial low rate seems to result from the time
5 required for the penetration of the substrate into the fibers since these fibers have a less porous morphology and are more hydrophobic. In the porous fibers (Type 2) the penetration of the substrate is barely hampered since the presence of PEG facilitates the wetting of the outer surface of the fibers and thereby allows the access of the aqueous substrate.

10 Altogether, these results demonstrate that enzymes attached to (e.g., encapsulated within) electrospun microtubes maintain their enzymatic activity.

Thus, the present technology overcomes both the problem of fiber dissolution and subsequent leaching of the enzyme and the exposure of the enzyme to harmful
15 solvents.

EXAMPLE 2

ELECTROSPUN MICROTUBES CAN RELEASE ENZYMES ATTACHED THERE TO

Experimental Results

20 ***Enzyme can be leached out of the electrospun microtubes*** - In order to determined whether the substrate diffuses into the fibers or the enzyme diffuses out of the fibers, 3 specimens containing alkaline phosphatase (AP) were cut out from the non-woven mat and placed in buffer. The buffer from the first sample was taken immediately and assayed (initial rinse). For the second and third samples the buffer was
25 taken and assayed after 24 hours and 72 hours, respectively. The first sample was tested in order to evaluate if any removable enzyme resides on the outer surface of the fibers after the spinning. The relative activity of the escaped enzyme in the buffers for the two types of fibers is presented in Figure 3. The results clearly indicate that a significant fraction of the enzyme has leached out of the fibers within the first 24 hours. Hence it
30 can be concluded that the reaction monitored for the mat (the electrospun microtubes) is a result of both in-fiber and out-fiber reactions. Interestingly however, for Type 1 fibers, which do not contain PEG in the shell, 82 % of enzyme has diffused outside the fibers

within 24 hours (compared to the mat). For Type 2 fibers, which are more hydrophilic due to PEG, the leaching of the enzyme is less although by no means negligible. The results also point out, as was already mentioned, that Type 2 fibers are the most active system and attains 55 % of the activity of the free enzyme.

5 ***Alkaline phosphatase can migrate to the outer surface of the microtubes during electrospinning*** - Moreover, it is clear that some enzyme is located on the outer surface of the fibers (i.e., attached to the microtube shell) and can immediately enter the surrounding buffer upon rinsing. The migration of the enzyme to the outer surface of the fibers during the spinning is more pronounced in Type 2 and is probably due to PEG
10 which might serve as hydrophilic conduit for the enzyme. It has been already found (Reznik SN, et al., 2006) that in the core-shell process all charges immediately accumulate at the outer surface of the shell in the drop. Ions are preferentially subjected to this migration as the electric field is applied. In the present case, the protein, which is a charged molecule, can also migrate to the outer surface during electrospinning. This
15 may explain the relatively large quantity of enzyme that is released by rinsing.

Altogether, these results demonstrate that type 2 fibers attain more enzymatic activity as compared to type 1 fibers. In addition, the enzyme within the type 2 fibers (which include PEG in the shell) is present in both the outer surface and the inner surface of the microtubes. Thus, these results demonstrate that the even though the
20 enzyme is mixed with the core solution, it is capable of migrating into the outer surface of the shell during the electrospinning process.

EXAMPLE 3

ELECTROSPUN MICROTUBES CAN SERVE AS MICROREACTORS

Experimental Results

25 ***β -galactosidase remains within the hollow fiber micro-reactor*** - The kinetics of the β -GAL reaction were determined as described hereinabove for AP and the results are presented in Figures 4a-b. Briefly, pieces of mat (electrospun microtubes) were immersed in buffer for a quick rinse, 24 or 72 hours to determine if there is any leaching
30 of the enzyme (Figure 5, shown are results of Type 2 as an example). Two striking differences between the AP and β -GAL series were observed (Figure 6): (i) the β -GAL reaction rate of the Type 1 mat is much slower relative to that found for AP, and the

activity of AP in the type 1 mat is slower relative to that found for the activity of the free enzyme in the core solution (prior to electrospinning). In type 2, conversely, the activity of the β -GAL is higher than that of AP and the reaction velocity of the β -GAL enzyme is comparable to the free enzyme. This intense response of Type 2 is attributed to the high porosity of these fibers and their hydrophilic nature; (ii) the results shown in Figure 5 for Type 2 fibers demonstrate that the β -GAL enzyme doesn't diffuse out of the fibers even after 72 hours even though Type 2 might be thought to be the more likely to allow the enzyme to escape. That is, the reaction takes place only within the fibers. Thus, for β -GAL, the hollow fibers act as a micro-reactor; the substrate which enters the "reactor" through the entire porous shell is cleaved by the encapsulated enzyme and the reaction product then diffuses out into the surrounding medium. Thus, without being bound by any theory it seems that the reduction in the reaction rate for Type 1 is related solely to the slow diffusion of the substrate into the fibers. The size of the enzyme seems to affect both the amount of enzyme that can migrate during the spinning to the outer surface of the fibers and its subsequent escape into the surrounding medium. In the case of β -GAL only a small amount of enzyme was detected in the rinsing buffer for Type 2 while for Type 1 no enzyme was detected at all (data not shown). As was argued before, in the fibers which do not contain PEG in the shell (Type 1) an additional moderate slope at the very beginning of the reaction can be observed (Figure 4b-inset, marked by dark arrow) due to the relatively high hydrophobicity of the surface which hinders the access of the aqueous dissolved substrate.

Altogether, these results demonstrate that the Type 1 system results in lowered enzymatic activity, especially for large proteins such as β -GAL, which cannot diffuse through the shell pores. The fact that Type 1 fibers are hydrophobic and non-porous and thus inhibit the entrance of these substrates seems to make Type 1 fibers less efficacious for their use as flow-through reactors. This is in contrast to the remarkably efficient system obtained with Type 2 fibers.

The enzymatic reaction occurs within type 1 microtubes (hollow fibers) - Visual evidence that the enzymatic reaction occurs within the type 1 fibers was obtained by using a substrate in which one of the products is fluorescent. For both AP and β -GAL enzymes the substrates used liberate 4-methyumbelliferone after hydrolysis which allows imaging by fluorescence microscopy. As is clearly seen in Figures 7a-b, the

interior of the fibers is fluorescent while the surrounding medium is dark. Thus, these results clearly show that both enzymatic reactions (of AP and β -GAL), in fact, take place within the fibers. It is important to emphasize that, in contrast to the mat immersion experiments, this method is very sensitive and enables the detection of very small amounts of product which accumulates within a relatively short time. Indeed, these images were acquired within 1-2 minutes after the substrate was applied, a time scale which is larger than the characteristic time [about 10 seconds, as was calculated by the present inventors (Dror Y., et al., 2007)] of the diffusion through the micro-tubes wall. Hence, these results are not in contradiction to those of the mat immersion experiments in which the kinetics were followed over a much longer period during which both the product and the enzyme (in the AP case) can diffuse outside the fibers. In Figure 7b short slugs (sections) of fluorescent liquid are observed. This phenomenon has been previously found in such fibers (Dror Y., et al., 2007).

The results shown in Examples 1-3 demonstrate the direct incorporation of enzymes into micro-tubes fabricated by co-electrospinning by introduction of the enzymes into the aqueous core solution of the microtube (e.g., PEO). The shell solution in this case was made of PCL dissolved in mixture of chloroform and DMF. The separation between the outer organic and inner aqueous phases was found to preserve enzyme activity during and after spinning when the electrospun fibers (mats) were subsequently placed in an aqueous environment.

Two types of micro-tubes were fabricated which differ in their shell morphology. Type 2 shells were produced by adding PEG to the shell solution. By using a mixture of PEG and PCL in the shell, pores were formed during the solidification process and this, in turn, directly affected the transfer of molecules into and out of the fibers. As a consequence, the more porous fibers (Type 2) exhibited higher rates of enzymatic reaction. In addition two enzymes differing in their molecular weight were incorporated: AP and β -GAL. The difference in the molecular weight between the enzymes was well reflected in the kinetics of the enzymatic reactions for both types of micro-tubes. While AP could diffuse outside the fibers, β -GAL remained in the fibers without any leaching of the enzyme and the progress of the reaction depended only on the arrival of the substrate from the surroundings. Thus, the AP fibers act as an enzyme release device in which the release rate can be tailored by modifying the morphology of the shell and, on

the other hand, the β -GAL fibers act as an enzymatic micro-reactor with an efficient provision of the substrate through the entire surface area and efficient discharge of the product. Thus, by manipulating the morphology of the shell, the substrate supply and product release rate can be controlled. This method of encapsulation can be used when a separation between the enzyme and an external aqueous environment is desired (e.g. with living tissue to avoid immunological reactions). The remarkable retention of the enzyme activity for β -GAL Type 2 fibers clearly demonstrates that this approach preserves the activity of the enzyme.

Another advantage of the core-shell fiber method is the small volume within the micro-tubes which enables the quick buildup of the product. This is important for enzymes working in sequence where the local concentration of the product of the first reaction serves as the substrate for a subsequent reaction. In this regard, these nanotubes are somewhat analogous to living cells except that any manner of enzymes may be added to the fibers without regard to their biological origin. Another advantage of this system is that unlike living cells, there is no discrimination as to which type of small molecules may enter these tubes. For example, phosphorylated molecules (like p-nitrophenyl phosphate) which do not enter *Escherichia coli* cells can enter the microtubes described herein.

EXAMPLE 4

USE OF THE ELECTROSPUN MICROTUBES AS MICROREACTORS FOR CHEMICAL TRANSITIONS

The microtubes of the invention as reactions for multi-step enzymatic processes - In order to synthesize or catabolize molecules which require multi-step enzymatic processes the present inventors have devised electrospun microtubes which include the enzymes participating in the multi-step enzymatic process, attached thereto, as follows.

For a biochemical pathway which involves the conversion of A to E via compounds B, C and D, the second polymeric solution for forming the coat over the internal surface of the shell (also referred to as a core solution) is mixed with the following enzymes: the enzyme that converts the starting substrate A to intermediate compound B, the enzyme that converts B to C, the enzyme that converts C to D, and the

enzyme that converts D to the end product E (see for example, Figure 9). It should be noted that due to the proximity of the enzymes to each other in the microtube (which can be a closed micro-reactor), the local concentrations of each of the intermediate molecules, *i.e.*, compounds B, C, and D is relatively high, which enables the kinetics of the reactions to occur, similarly to their concentrations in cells or cell compartments (e.g., mitochondria). The shell solution is made from hydrophobic polymers (water insoluble polymers), with or without the addition of PEG.

Thus, the microtube of the invention enables higher local concentrations of intermediate compounds which can not be reached from the same starting material (substrate A) if an open system (such as any solid substrate to which an enzyme is immobilized) is used.

The creation of micro- and nano-fibers containing enzymes simulates the cellular structure because two or more different enzymes involved in a particular synthesis or degradation can be put into proximity of one another. The interior of the tube is quite parallel to that of cells except that the borders of the tube are made from a water insoluble substance whereas living cells are encompassed by lipid membranes. In addition the microtubes are much longer than cells but quite similar in other dimensions to a bacterial cell. In the electrospun fibers, any small molecule can pass through the water insoluble barrier (pores can be made) regardless of its chemistry with the only provision that the small molecule be water soluble.

Thus, the present technology allows the entrapment of high concentrations of an enzyme or several enzymes within a confined space. Single or multi-step reactions can then take place where the product of one reaction is the substrate of a second and the second enzyme is spatially nearby. While such multi-step reactions can occur in an open system, the time necessary to reach the end product is orders of magnitude greater than within the microtube of the invention.

The microtubes of the invention can include enzymes from different species -
Another very important advantage of these electrospun fibers is that there is no limitation of which enzymes can be embedded. In nature, cells contain enzymes useful for their growth and reproduction. Organisms have not been designed or selected for industrial processes desired by humans. The microtubes of the invention allow any desired combination of enzymes to be brought together. This might mean that enzymes

from totally different organisms (e.g. flies and humans) could be placed together for some use while in nature no organism exists with this combination.

The microtubes of the invention as micro-reactors for the production of molecules which are intermediate compounds of a natural process - The enzymes

5 encapsulated might be those carrying out part of pathway making the end product a substance that is usually an intermediate molecule in living organisms. This allows one to synthesize molecules that cannot be obtained in any amount from living material because the concentration of intermediates in cells is usually very low (in the order of 10 μ M or less). For example, to synthesize indole-glycerol phosphate which is an
10 intermediate in tryptophan synthesis within the cells of lower organisms, the enzymes that participate in the conversion of anthranilate (an inexpensive compound) to indole-glycerol phosphate should be included in the microtube, while the enzymes that continue the synthesis of tryptophan from indole-glycerol phosphate are excluded from the microtube. In summary, many combinations of enzymes from different organisms may
15 be put together without any genetic engineering and partial sets of enzymes can also be used. The number of possible useful combinations is therefore very large.

Thus, the microtubes of the invention can be used as enzymatic micro-reactors where the inner space enables a confined but free reaction space. The substrate diffuses through the shell to the inner space where the enzymatic reaction takes place and the
20 product can then diffuse out.

EXAMPLE 5

THE ELECTROSPUN MICROTUBES AS BIOSENSORS

Since the electrospun microtubes of the invention are insoluble in aqueous
25 solutions, they can provide an excellent tool for the construction of biosensors.

Since any enzyme or combination of enzymes can be encapsulated in the electrospun microtubes, a variety of biosensors can be devised. For example, enzymes that are sensitive to heavy metals exhibit loss of activity in the presence of heavy metals. Another example, firefly luciferase, for example can be electrospun with its luciferin
30 cofactor and any reaction affecting ATP production can be used in conjunction with light output the signal.

EXAMPLE 6**THE ELECTROSPUN MICROTUBES FOR FLOW-THROUGH APPLICATIONS**

Water purification or detoxification - The electrospun microtubes of the invention, which are made of a water-insoluble outer shell, enable the flow of liquids. It will be appreciated that enzymes embedded in such microtubes can be used to purify the liquid flowing past the microtubes as molecules diffuse in and out of them.

Thus the present inventors have devised water purification or detoxification apparatuses, as follows. The second polymeric solution which forms the coat over the internal surface of the shell (also referred to as a core solution) [which is made of water-soluble polymer(s)] includes enzymes which remove a toxic moiety from water, such as the gene product of the *atzA* gene from *Pseudomonas* ADP that removes the chlorine from atrazine, a toxic substance. The shell solution [which is made of water-insoluble polymer(s)] is designed so as to enable water flow within the microtube. The effluent would thereby be rendered free of atrazine and safe for animal and human consumption.

Dialysis - The microtubes of the invention can be used in various applications which remove certain compounds, such as dialysis procedures on humans. Thus, the electrospun microtubes can be made using a shell polymer which prevents the diffusion of enzymes therethrough, yet enables passage of water and substances that need to be purified. It will be appreciated that such microtubes can be also implanted into a subject in need thereof (e.g., a subject in need of dialysis), and due to the structure of a closed conduit, which prevents passage of embedded enzymes through the shell, there is no immune response to the implanted conduit.

EXAMPLE 7**THE ELECTROSPUN MICROTUBES FOR ENZYME THERAPY**

Since the electrospun microtubes of the invention are insoluble in aqueous solutions, they should provide excellent tool for the construction of material for enzyme therapy. Some individuals lack certain enzymes, usually as a result of their being homozygous for recessive alleles that lead to synthesis of an inactive enzyme. Gene therapy attempts to introduce the missing active gene which thereby leads to the production of an active enzyme. However, this technique is still quite inefficient. A different way of treating such patients is enzyme therapy, in which the missing enzyme

is exogenously supplied to the subject. The main drawbacks of the second method is that injection of enzymes often leads to the formation of antibodies against them and the half-life of the enzymes within the body may be quite short.

Thus, the present inventors have devised an apparatus for enzyme therapy, as follows. Briefly, the electrospun microtubes which include a water-insoluble shell can include enzymes which are needed for enzyme therapy, and be further implanted in a subject in need thereof.

Enzyme therapy for PKU - Phenylketonuria (PKU) occurs in slightly less than 1 per 10000 individuals and is an autosomal recessive genetic disease caused by homozygosity of alleles encoding defective enzymes. Phenylalanine and tyrosine are amino acids that are found in most proteins. In humans, the source of these two amino acids is dietary protein. In normal individuals, excess phenylalanine is converted to tyrosine. Excess tyrosine, in turn, is broken down to fumarate and acetoacetate. Both tyrosine and phenylalanine are essential for human protein synthesis. In addition, tyrosine is the precursor of melanin (skin and eye pigment) and for certain hormone like substances such as thyroxine. Phenylketonuria is caused by the lack of the enzyme (phenylalanine 4-monooxygenase EC 1.14.16.1) that converts phenylalanine to tyrosine. The result of this defect is the accumulation of phenylalanine in the blood along with a number of compounds that are derived from it (e.g. phenylpyruvic acid and phenyllactic acid). The result is brain damage (and an IQ of 30-70) as some of these compounds are toxic. The current method of preventing deterioration of the disease is to limit the intake of phenylalanine. The present inventors have envisaged that PKU can be treated by implanting electrospun microtubes containing the missing enzyme, phenylalanine hydroxylase (PAH; GenBank Accession No. NP_00026), in a subject diagnosed with PKU, and thereby enabling the breakdown of excess phenylalanine in the subject. It will be appreciated that in this case, the microtube can be designed so as to enable release of enzyme from the inner surface through the outer shell (e.g., using PEG in the outer shell) or alternatively can be designed such that the enzyme is entrapped (or remains) within the microtubes and effects its activity there (e.g., by diffusion of the substrate or end-product through the shell pores, or microtube opening(s)). As phenylalanine hydroxylase can be phosphorylated (with a molecular weight of consists of 50,000 Da) or dephosphorylated (with a molecular weight of 49,000 Da), the size of the pores in the

shell should enable passage (by diffusion) of each of these forms (e.g., about 5 nm in diameter). Microtubes can be made with smaller pores that will prevent the loss of the enzyme which will remain within the microtube.

5

EXAMPLE 8

THE DESORPTION PROCESS

Figure 8 schematically depicts the desorption of a molecule-of-interest to the microtube of the invention. After the electrospinning process, the molecule-of-interest (e.g., a protein, an enzyme) is adsorbed to the inner side of the microtubes. As mentioned, the porosity of the microtubes can be controlled (e.g., adding PEG to the shell polymer), therefore the shell consists of nanopores (see #3 in Figure 8) with an opening to the outer surface of the microtube, which herein the pores are considered to have a cylindrical shape. When immersing the microtubes in a solution (e.g., a tissue culture medium, a physiological solution or any buffer) most nanopores opening are accessible to the solution.

Once the microtubes are immersed in the solution the nanopores are filled by the solution through capillary rise (see arrow # 1 in Figure 8). It will be appreciated that the time of the capillary rise depends on the solution rheological properties (viscosity and surface tension), the wetting angle and the geometry of the nanopore (length and radius). The solution penetrates to the microtube and start wetting and filling its entire inner volume. Desorption of the molecule-of-interest from the microtube wall depends mainly on the rate of the release of the molecule-of-interest from the polymer of the second polymeric solution. Finally, the molecule-of-interest (e.g., protein/enzymes) diffuses (see arrow # 2 in Figure 8) into the solution and released to the surroundings of the microtubes (assuming that the major release is through the microtubes envelope).

Note that the geometry (radius and length) of the nanopore is controllable, by adjusting the shell thickness, or by blending, more PEG to the shell polymer. Therefore, the release from or confinement in the microtube of the invention is controllable, e.g., in certain cases the molecule-of-interest is released from the microtube, whereas in other cases, the molecule-of-interest remains in the inner volume of the microtube. As shown in Figure 2a, a molecule-of-interest is released from the microtube of the invention in a

controlled manner, which can be extended beyond 2500, minutes, e.g., for several days and months.

5 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.

10 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
15 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 molecule-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 molecule-of-interest, thereby attaching the molecule-of-interest to the microtube.
2. A microtube comprising an electrospun shell, an electrospun coat over an internal surface of said shell and a molecule-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(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 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 % (w/w) 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 molecule-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 molecule-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 2-7, 9-17, wherein said molecule-of-interest comprises a polypeptide, a polynucleotide, a carbohydrate, a small molecule, or any combination thereof.

19. The method of any of claims 1, 7-18 or the microtube of any of claims 2-7, 9-18, wherein said molecule-of-interest comprises a member of an affinity pair.

20. The method or the microtube of claim 18 or 19, wherein said polypeptide is an enzyme.

21. The method or the microtube of claim 20, wherein said enzyme is alkaline phosphatase (SEQ ID NO:1) or beta-galactosidase (SEQ ID NO:2).

22. The method of any of claims 1, 7-21 or the microtube of any of claims 2-7, 9-21, wherein said first polymeric solution comprises polyethylene glycol (PEG).

23. The method of any of claims 1, 7-22 or the microtube of any of claims 2-7, 9-22, wherein said shell comprises pores.

24. The method of any of claims 1, 7-16, 18-23 or the microtube of any of claims 2-7, 9-16, 18-23, wherein said shell prevents diffusion of the molecule-of-interest therethrough.

25. A method of processing a substrate-of-interest, comprising contacting the substrate-of-interest with the microtube of any of claims 2-7, 9-24, wherein said molecule-of-interest is capable of processing said substrate, thereby processing the substrate-of-interest.

26. The method of claim 25, wherein said processing said substrate-of-interest comprises incorporating said substrate-of-interest in a synthesis reaction catalyzed by said molecule-of-interest.

27. The method of claim 25, wherein said processing said substrate-of-interest comprises incorporating said substrate-of-interest in a catabolism reaction catalyzed by said molecule-of-interest.

28. A method of depleting a molecule from a solution, comprising contacting the solution with the microtube of any of claims 19-24, wherein said member of said

affinity pair is selected capable of binding said molecule, thereby depleting the molecule from the solution.

29. The method of claim 28, further comprising collecting said solution following said contacting.

30. The method of claim 28, wherein said solution comprises blood.

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

(a) contacting the solution with the microtube of any of claims 19-24 under conditions which allow binding of the molecule to the microtube via said member of said affinity pair which is selected capable of binding said molecule, and;

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

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

(a) contacting the sample with the microtube of any of claims 19-24, wherein said member of said affinity pair is selected capable of binding said molecule, and;

(b) detecting binding of said molecule by said member of said affinity pair;
thereby detecting the presence of a molecule in the sample.

33. The method of claim 28, 29, 31 or 32, wherein said affinity pair is selected from the group consisting of an enzyme and a substrate, a hormone and a receptor, an antibody and an antigen, a polypeptide and a polynucleotide, a polynucleotide and a cognate polynucleotide, a polypeptide and a metal ion, a polypeptide and a carbohydrate.

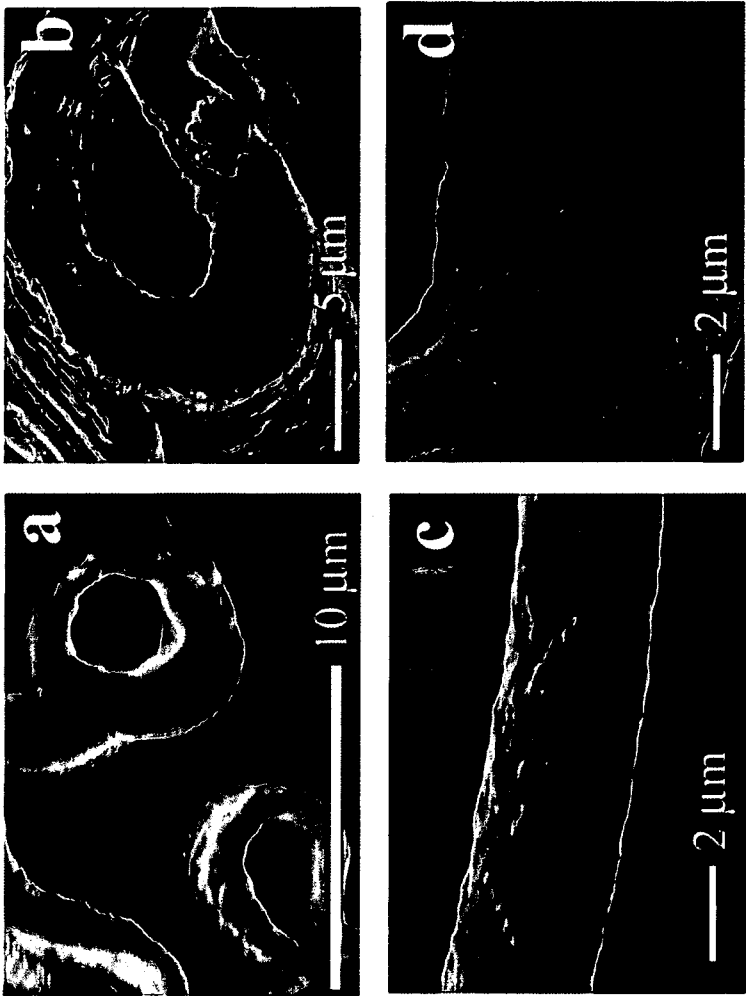
34. A method of releasing a molecule-of-interest to cells of a subject in need thereof, comprising implanting in the subject the microtube of any of claims 2-7, 9-23, to thereby release the molecule-of-interest to cells of the subject.

35. The method of claim 34, wherein a therapeutically effective amount of the molecule-of-interest is capable of treating a pathology in the subject.

36. The method of claim 34, wherein the molecule-of-interest comprises a polypeptide, and whereas a therapeutically effective amount of said polypeptide is capable of treating a pathology in the subject.

37. The method of claim 36, wherein said pathology is selected from the group consisting of a metabolic disorder, an endocrine disease, an autoimmune disease, and cancer.

38. The method of claim 36, wherein said polypeptide is selected from the group consisting of insulin (SEQ ID NO:6), phenylalanine hydroxylase (PAH) (SEQ ID NO:3), dystrophin (SEQ ID NO:4), beta-glucosidase (GBA) (SEQ ID NO:5), and ceruloplasmin ferroxidase (CP) (SEQ ID NO:7).



FIGs. 1A-D

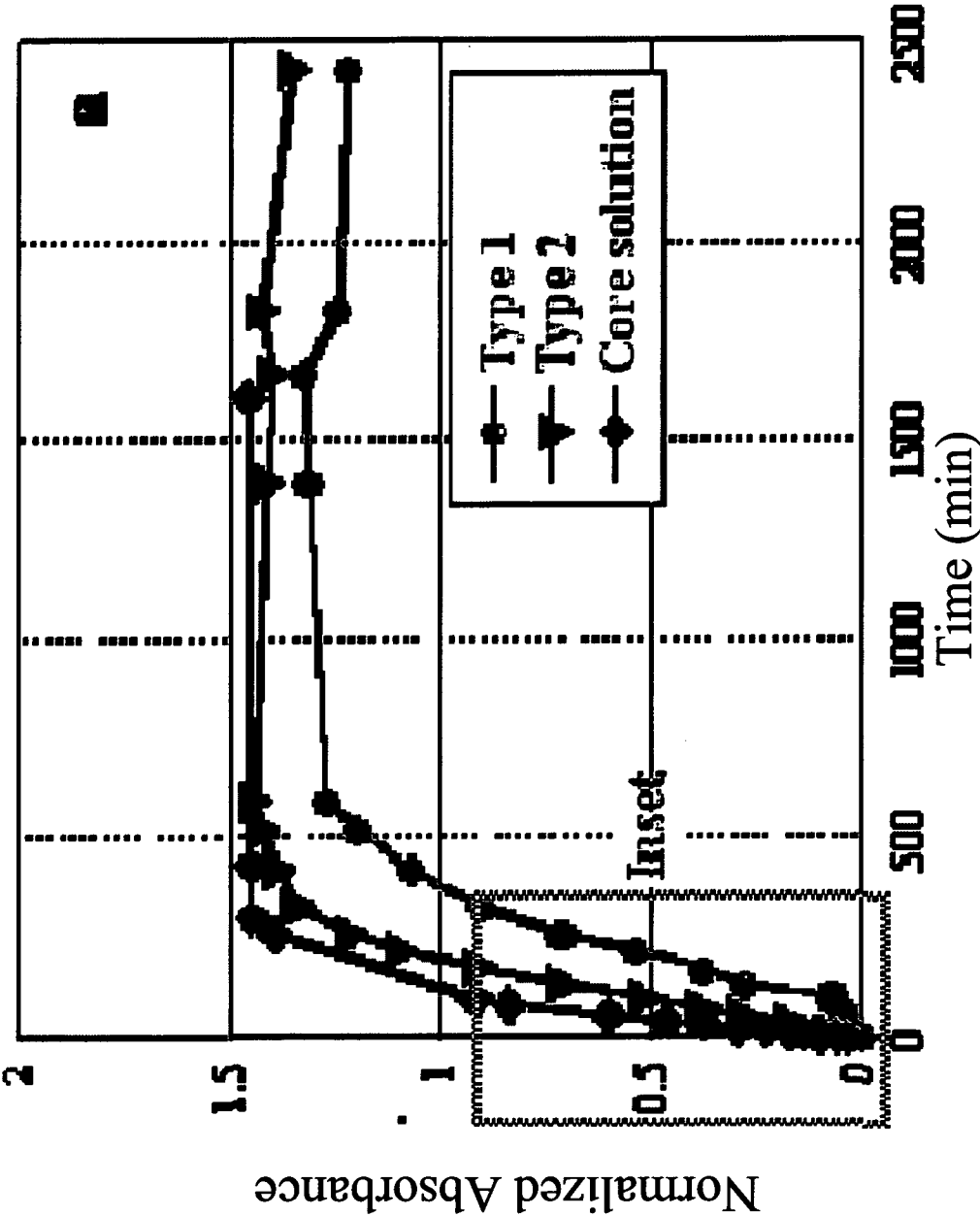


FIG. 2A

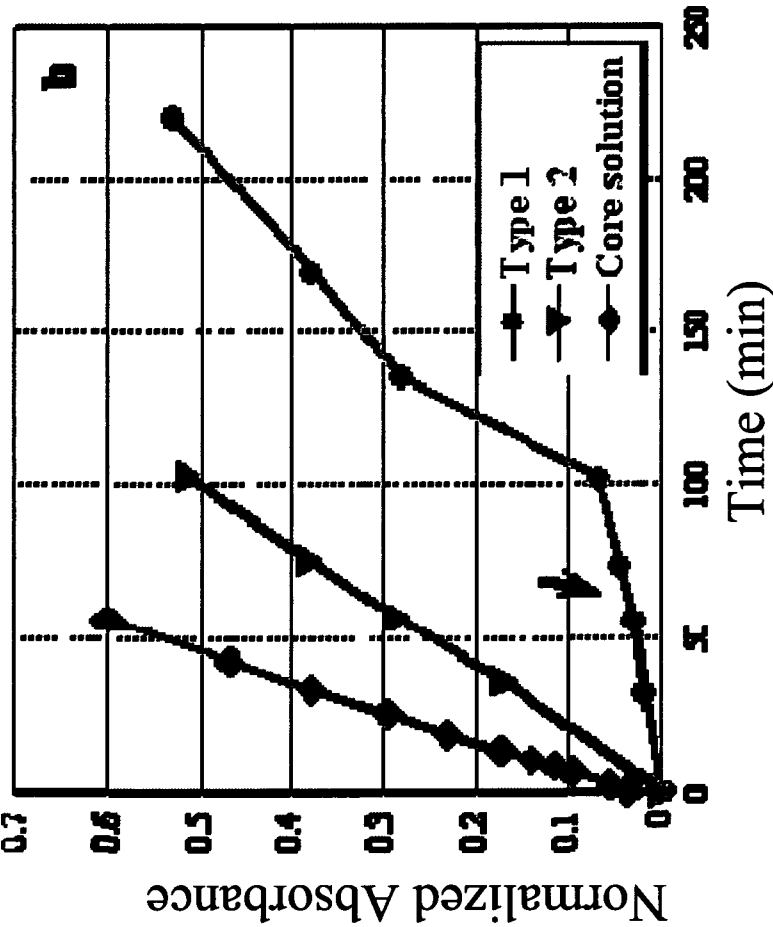


FIG. 2B

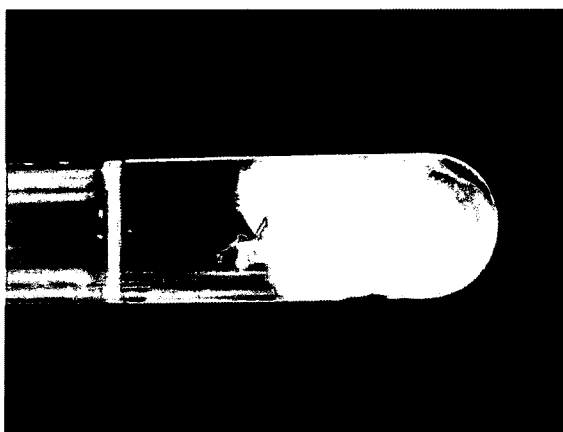


FIG. 2C

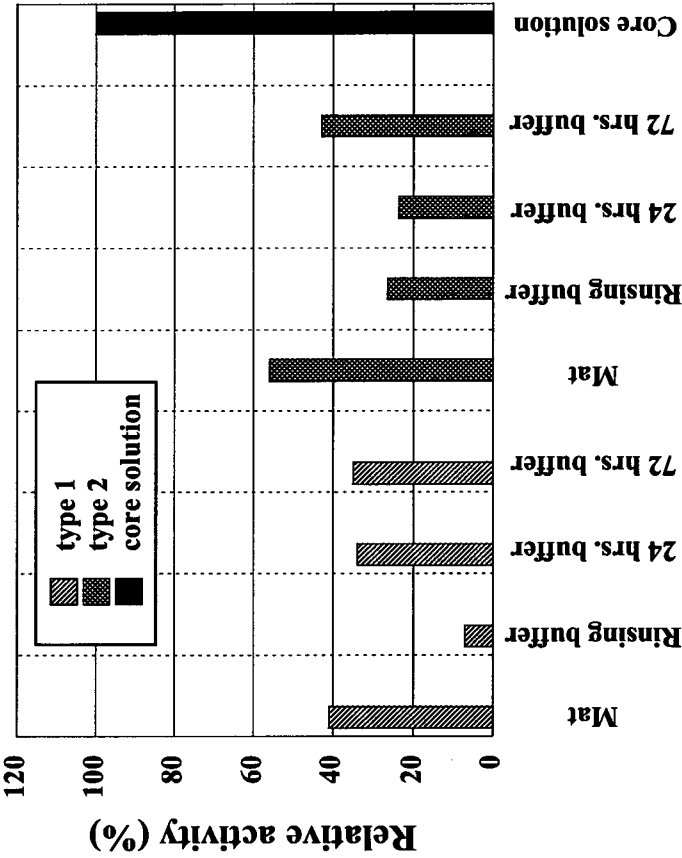


FIG. 3

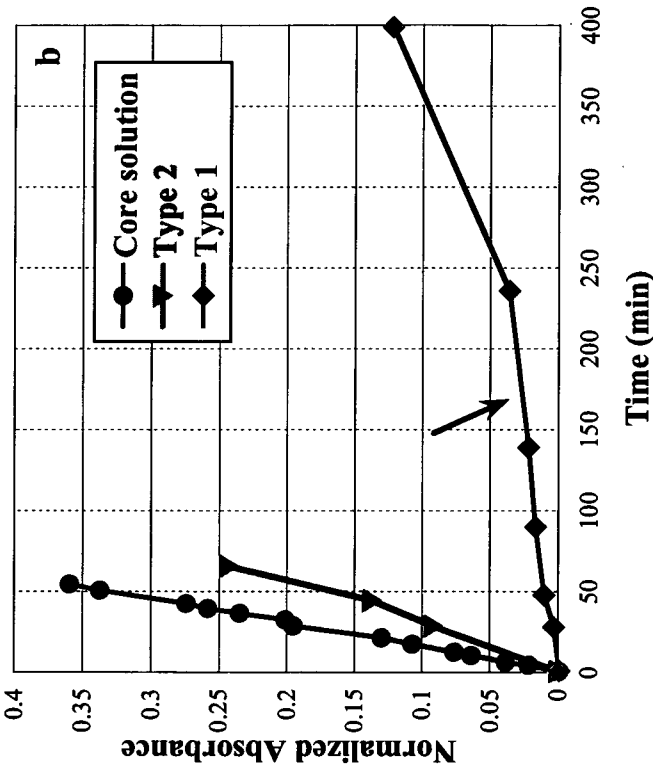


FIG. 4B

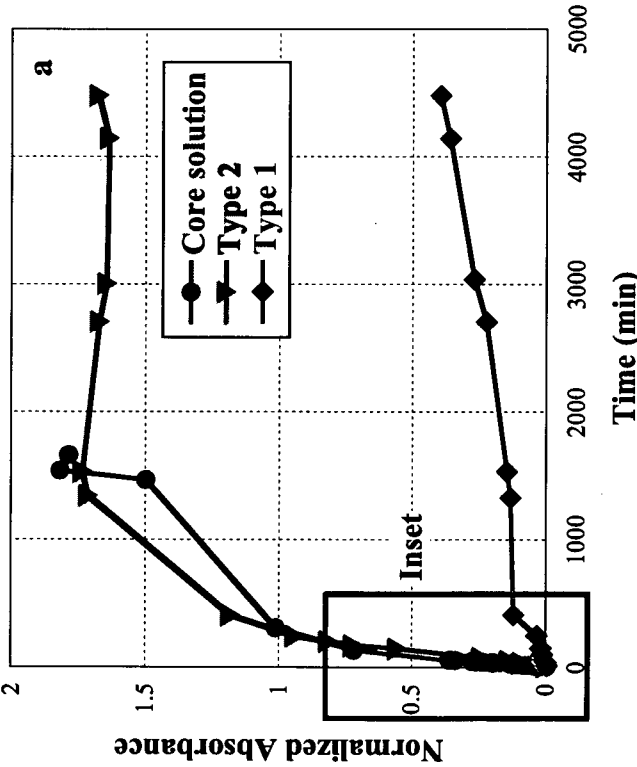


FIG. 4A

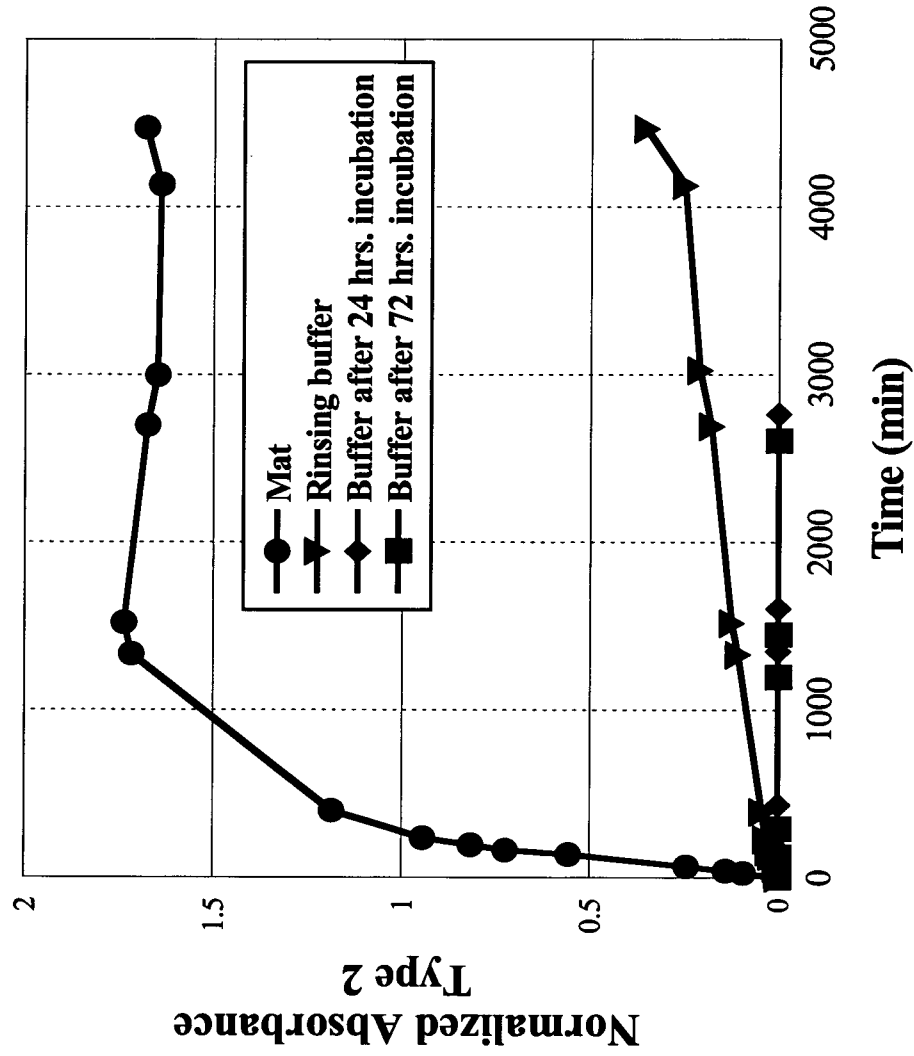


FIG. 5

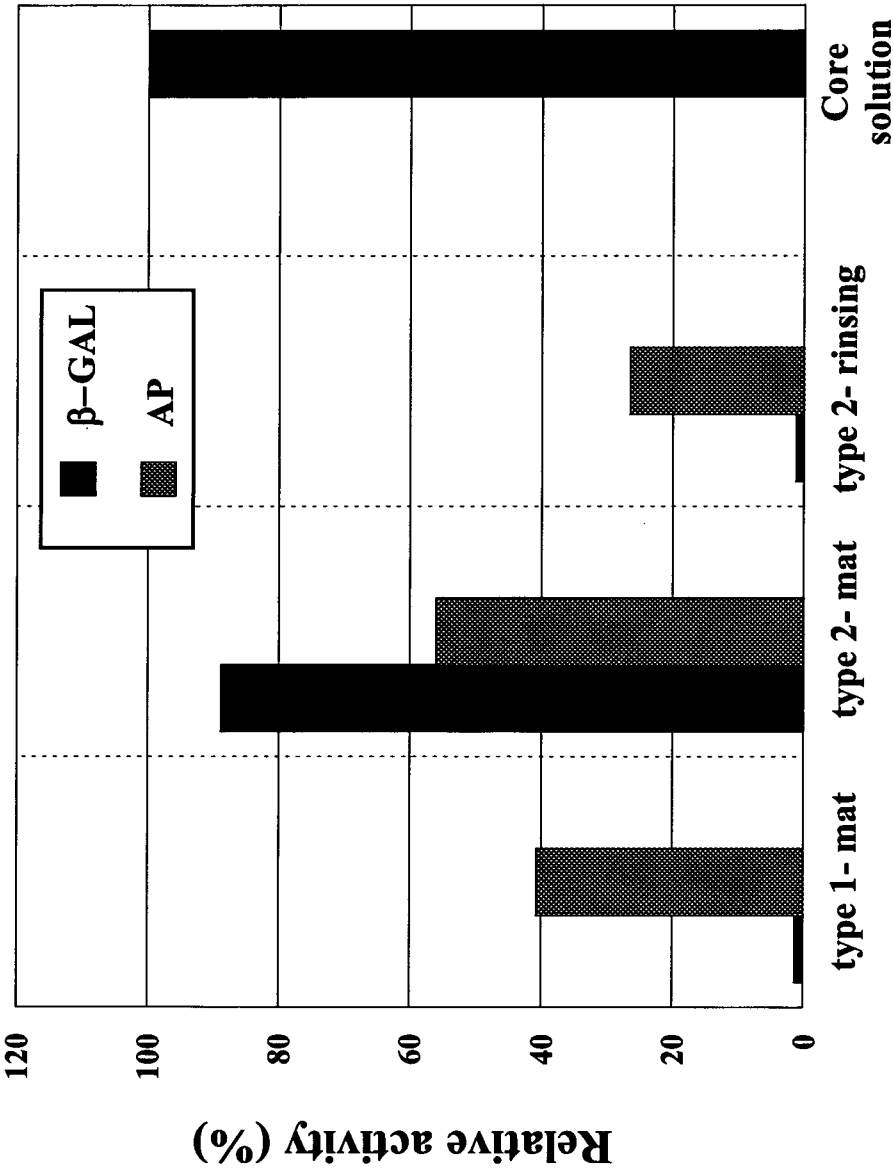


FIG. 6

FIG. 7A



FIG. 7B



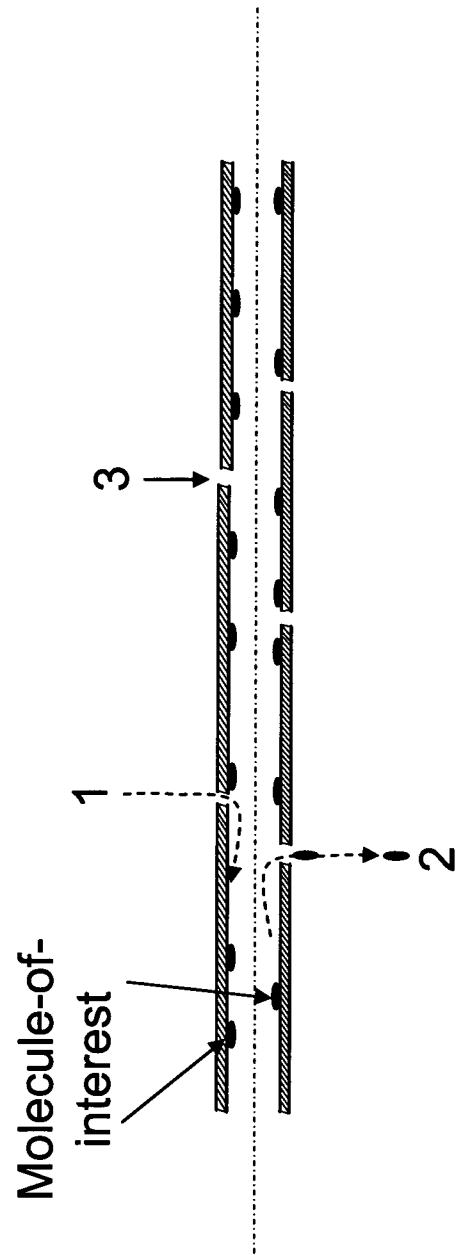


FIG. 8

Multiple Enzyme Pathways

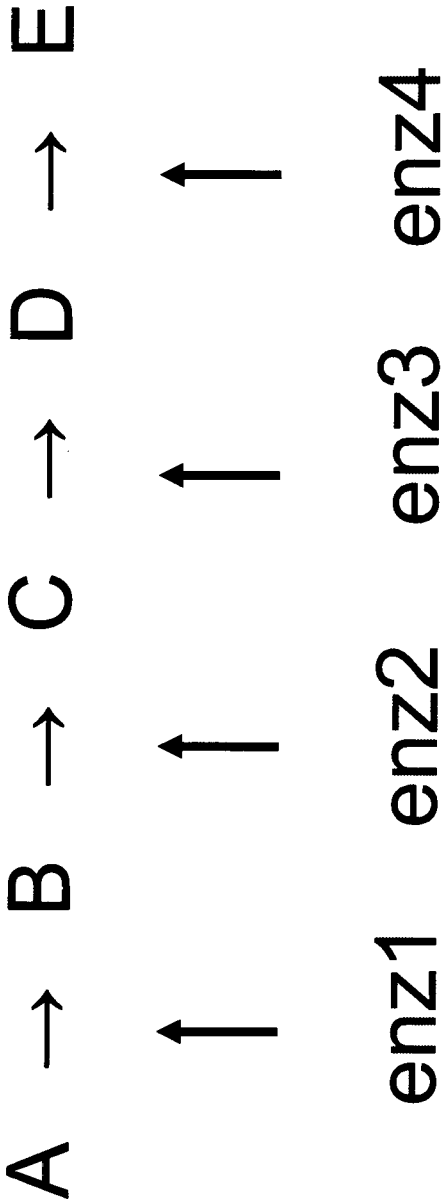


FIG. 9

12/12

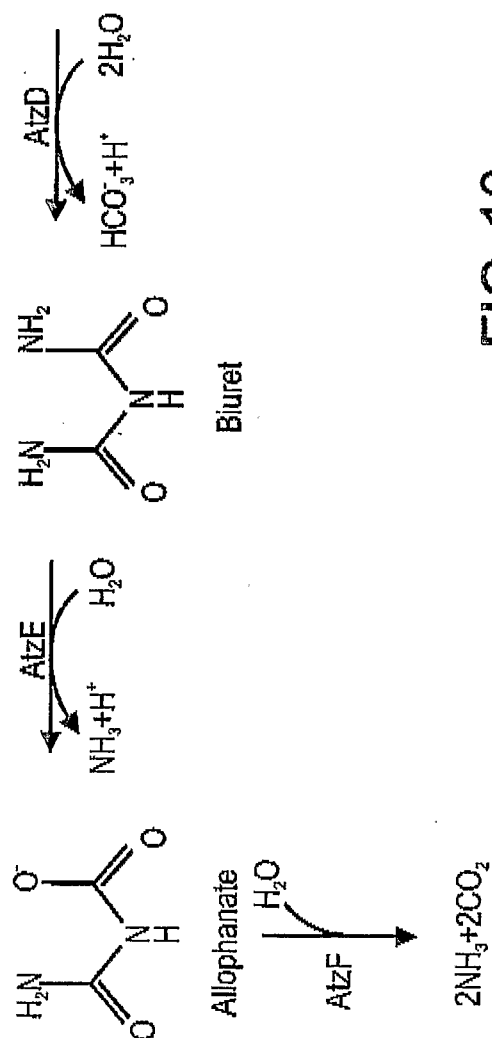
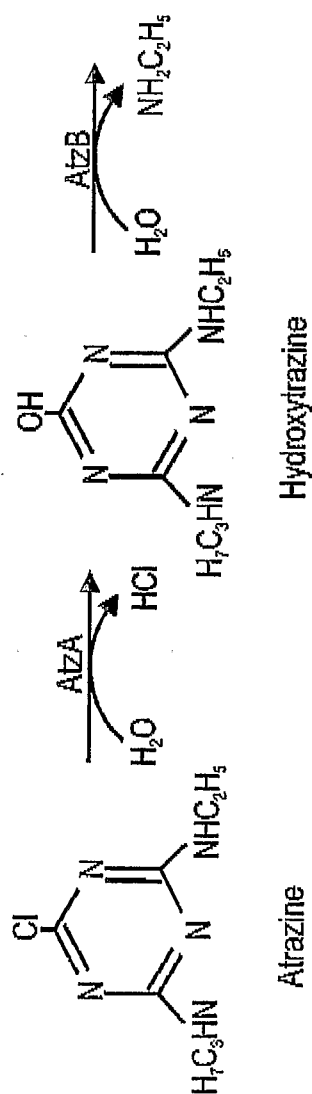
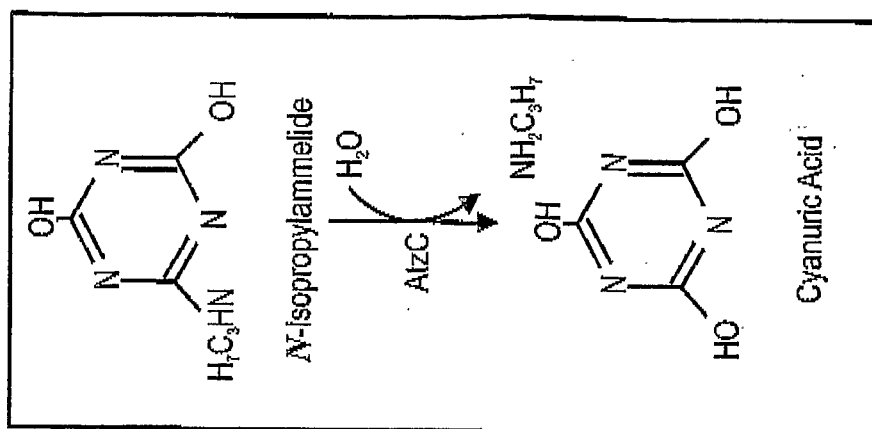


FIG. 10