

[Monitor Patents](#) - Methods and products for delivering biological molecules to cells using multicomponent nanostructures

FreshPatents.com
Track New Patents and Technologies



monitor



location



industry



inventors



agents

Title/Abstract

FreshPatents Search



How **KEYWORD MONITOR** works... a **FREE** service from FreshPatents

1. [Sign up](#) (takes 30 seconds).
2. [Fill in the keywords](#) to be monitored. (i.e. **aliasger salem**)
3. Each week you receive an email with patent applications related to your keywords. [Start now!](#)

[05/12/05](#) | [#20050101020](#) | **Browse Patent Applications:** [Prev](#) - [Next](#) | Browse Industry: [USPTO Class 435](#)

Methods and products for delivering biological molecules to cells using multicomponent nanostructures

This invention is predicated on the present applicants' discovery that nanostructures comprising discrete regions of different composition can be used to deliver to a biological cell a desired combination of molecules in close proximity. Different molecules can be selectively bonded to discrete regions of different composition in sufficiently close physical relationship to enhance delivery or effectiveness within the cell. The preferred nanostructures are multicomponent nanorods. Important applications include delivery of missing DNA sequences for gene therapy and delivery of antigens or DNA encoding antigens for vaccination.

Agent: [Docket Administrator Lowenstein Sandler PC](#) - Roseland, NJ, US

Inventors: [Aliasger Karimjee Salem](#), [Kam W. Leong](#), [Peter Charles Searson](#)

Class: [435459000](#) (USPTO), [C12N015/85](#) (Intl Class)

Brief Patent Description - [Full Patent Description](#) - [Patent Application Claims](#)

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No. 60/482,141 filed by Dr. Aliasger K. Salem et al on Jun. 24, 2003 and entitled "Multifunctional Nanorods for Gene Delivery", which is incorporated herein by reference.

FIELD OF THE INVENTION

[0003] This invention relates to methods of delivering biological molecules to cells and, in particular, to methods of delivering to cells a desired combination of biological molecules in close physical proximity. It also includes products for effecting such delivery.

BACKGROUND OF THE INVENTION

[0004] The capability of delivering biologically active molecules to plant and animal cells is of great importance to medicine and genetic research and engineering. In medicine, for example, the development of effective vaccines requires systems for providing characteristic portions of infectious biological entities to immune system cells so that the immune system will recognize and fight an infection. When such characteristic portions (antigens) of entities such as viruses, bacteria or even tumors are appropriately provided, the immune systems identifies the antigens as foreign and stimulates development of immunological countermeasures. One way to provide antigens is to deliver them directly into cells. Another way is to deliver to the cells DNA sequences that encode the antigens.

[0005] Gene therapy seeks to introduce additional genetic material (typically DNA) into a cell in such a way that the additional genetic material will be functionally incorporated into the existing genetic material of the cell. For example, there are certain diseases that are caused by the absence in cells of normally present DNA sequences (genes) needed to make critical proteins. Gene therapy seeks to alleviate such diseases by providing the cells with the missing DNA sequences so that the cells themselves can provide the critical proteins. To achieve this goal, the missing DNA sequences need to be introduced into cells in such a fashion that they are functionally incorporated into the genetic material and mechanisms of the cells.

[0006] The effectiveness of an active biological molecule in a cell often can be enhanced by the presence of one or more additional different molecules. For example, there are molecules, called adjuvants, that will increase the likelihood that an antigen will be recognized as an appropriate target for immunological countermeasures. As another example, there are also molecules that will interact with cell receptors and increase the likelihood of incorporation into the cell. Such enhancing molecules, however, typically must be close to the active molecule in order to enhance its effectiveness.

[0007] Conventional approaches to delivering biological molecules to cells leave much to be desired. The common approach to gene therapy is based on the fact that viruses have evolved to inject genetic material into a cell and use the cell's genetic machinery to replicate the viral genetic material. Appropriate modification of the virus might eliminate its harmful features and redirect a viral vector to deliver desirable genetic material into the cell. However virus vectors often generate counterproductive host immune responses and present a risk of killing infected host cells (cytotoxicity).

[0008] Other delivery approaches that have been suggested include the use of carriers comprising liposomes, polymers and gold nanoparticles. They have not, however, achieved notable success in efficiently incorporating new genetic material or in making more effective vaccines. Accordingly there is a need for improved methods and products for delivering biological molecules to cells.

SUMMARY OF THE INVENTION

[0009] This invention is predicated on the present applicants' discovery that nanostructures comprising discrete regions of different composition can be used to deliver to a biological cell a desired combination of molecules, including at least one biological molecule, in close proximity. Different molecules can be selectively bonded to discrete regions of different composition in sufficiently close physical relationship to enhance delivery or effectiveness within the cell. The preferred nanostructures are multicomponent nanorods. Important applications include delivery of missing DNA sequences for gene therapy and delivery of antigens or DNA encoding antigens for vaccination, and simultaneous delivery of interacting medicines in specific proportion and close proximity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The advantages, nature and various additional features of the invention will appear more fully upon consideration of the illustrative embodiments now to be described in detail in connection with the accompanying drawings. In the drawings:

[0011] FIG. 1 is a schematic block diagram of a method of delivering biological molecules to cells in accordance with the invention.

[0012] FIG. 2 is a schematic diagram illustrating functionalization of multicomponent nanostructures. In FIG. 2a nanorods are incubated with the 3-[(2-aminoethyl)dithio]propionic acid (AEDP) linker. The carboxylate end group binds to the nickel segment. The disulfide linkage at the center acts as a cleavable point within the spacer promoting DNA release within the reducing environment of the cell. In FIG. 2b plasmids are bound by electrostatic interactions to the protonated amines presented on the surface of the nickel segment. In FIG. 2c calcium chloride compacts the plasmids encoding the luciferase or GFP reporter genes; and in FIG. 2d rhodamine-conjugated transferrin presenting sulfhydryl groups is selectively bound to the gold portion of the nanorods.

[0013] FIG. 3 shows microphoto images pertaining to functionalized multicomponent nanostructures. FIG. 2a is a visible light image of dual functionalized 200 nm long Au/Ni nanorod. FIG. 3b fluorescence image of the rhodamine-tagged (543/570 nm) transferrin on the Au segment. FIG. 3c is a fluorescence image of the Hoechst stained (350/450 nm) plasmids on the Ni segment; and FIG. 3d is a fluorescent overlay image combining FIGS. 3b and 3c.

[0014] FIG. 4 shows microphoto images of cells transfected in accordance with the method of FIG. 1. FIG. 4a presents stacked laser scanning confocal microscope images of a live HEK293 cell (red/633 nm, green/543 nm). Rhodamine (633 nm) identifies the sub-cellular location of the nanorods whilst GFP expression (543 nm) provides confirmation of transfection throughout the cell. FIGS. 4b and 4c are, orthogonal sections that confirm the nanorods are within the cell. FIG. 4d shows confocal microscope stacked images, of a live HEK 293 cell stained with LysoTracker Green identifying the location of the nanorods (Rhodamine) in relation to acidic organelles in both orthogonal sections (FIGS. 4e and 4f).

[0015] FIG. 5 presents scanning electron microscope images of cells transfected in accordance with the method of FIG. 1. FIG. 5a is a SEM image of HEK293 cells after 1h incubation with 200 nm Au/Ni nanorods. FIG. 5b is a back-scattering SEM image of 200 nm Au/Ni nanorods after 4 h incubation showing the nanorods beneath the surface of the cell. FIG. 5c is a TEM cross-sectional image showing the intra-cellular location of the nanorods after 4 h incubation, and FIG. 5d is a, SEM image of 200 nm long nanorods after 4 h incubation.

[0016] FIG. 6 is a set of histograms summarizing results of transfection experiments. FIG. 6a shows percentage of GFP expression (area of cells fluorescing/total cell area) and FIG. 6b shows luciferase expression of: 1. nanorod-plasmid complex, 2. nanorod-plasmid/transferrin complex, 3. nanorod-plasmid/transferrin complex incubated with 100 micromoles chloroquine, 4. Lipofectamine (positive control) and 5. naked DNA (negative control).

[0017] FIG. 7 is a graphical illustration of ovalbumin-specific antibody responses in C57BL/6 mice immunized with various antigen or plasmid nanorod and gold particle formulations. C57BL/6 mice were immunized with control plasmid (no insert) bound to nanorods, ovalbumin antigen-nanorod formulation, ovalbumin antigen-gold particle formulation, pcDNA3-OVA7-nanorod formulation, pcDNA3-OVA7-gold particle formulation and ovalbumin antigen/control pcDNA3 (no insert)-nanorod

formulation via a gene gun. Serum samples were obtained from immunized mice 21 days after the initial vaccination. The presence of the ovalbumin-specific antibody was detected by ELISA using serial dilution of sera. The results from the 1:1000 dilutions are presented showing the mean absorbance (A450 nm) \pm SE.

[0018] FIG. 8 graphically illustrates ovalbumin-specific CD8⁺ T-cell precursors in C57BL/6 mice immunized with various antigen or plasmid-nanorod and gold particle formulations. C57BL/6 mice were immunized with control plasmid (no insert) bound to nanorods, ovalbumin antigen-nanorod formulation, ovalbumin antigen-gold particle formulation, pcDNA3-OVA7-nanorod formulation, pcDNA3-OVA7-gold particle formulation and ovalbumin antigen/control pcDNA3 (no insert) nanorod formulation via a gene gun. For vaccinated mice, 2 μ g of DNA or antigen/mouse were given twice. Splenocytes were harvested 7 days after the last DNA/antigen vaccination. Flow cytometry analysis: Splenocytes from vaccinated mice were cultured in vitro with the ovalbumin antigen overnight and were stained for both CD8 and intracellular IFN- γ . The number of IFN- γ secreting CD8⁺ T-cell precursors in mice immunized with antigen or plasmid-nanorod and gold particle formulations were analyzed by flow cytometry. The number of CD8⁺ IFN- γ double-positive T cells in 3 times 10⁵ splenocytes are represented by the quadrant in the upper right corner.

[0019] FIG. 9 schematically illustrates formation of three component nanowires.

[0020] FIG. 10 schematically shows a general approach for selective derivatisation of Au/Ni/Pt nanowires; and

Continue reading...

[Full patent description for Methods and products for delivering biological molecules to cells using multicomponent nanostructures](#)

Brief Patent Description - [Full Patent Description](#) - [Patent Application Claims](#)

Click on the above for other options relating to this Methods and products for delivering biological molecules to cells using multicomponent nanostructures patent application.

Related - 20050101019 - ([abstract](#)) - Method and device for targeted delivery of materials to selected single cells

###

 How **KEYWORD MONITOR** works... a **FREE** service from FreshPatents

1. [Sign up](#) (takes 30 seconds).
2. [Fill in the keywords](#) to be monitored. (i.e. **aliasger salem**)
3. Each week you receive an email with patent applications related to your keywords.

Start now! - Receive info on patent apps like Methods and products for delivering biological molecules to cells using multicomponent nanostructures or other areas of interest.

###

Previous Patent Application:

[Method and device for targeted delivery of materials to selected single cells](#)

Next Patent Application:

[Filtration container](#)

Industry Class:

[Chemistry: molecular biology and microbiology](#)

[Advertise on FreshPatents.com](#): Reach technology professionals, inventors, patent attorneys and more!

Design/code © 2004-2005 Freshpatents.com. [Website Terms and Conditions](#)

Patent data source: United States Patent and Trademark Office

Information published here is an abstract for research/educational purposes.

Complete official applications are on file at the USPTO and may contain additional data/images.

Thank you for viewing the *Methods and products for delivering biological molecules to cells using multicomponent nanostructures* patent info.

[IP-related news and info](#)

Results in 0.23738 seconds

aliasger salem Other interesting Feshpatents.com categories:

[Canon USA](#), [Celera Genomics](#), [Cephalon, Inc.](#), [Cingular Wireless](#), [Clorox](#), [Colgate-Palmolive](#), [Corning](#), [Cymer](#),