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ABSTRACT

The invention concerns a nanofiber structure with immobilized organic agents that consists of silica nanofibers whose surface was modified with aminoalkylalkoxysilane and of subsequently immobilized organic agents. The invention also concerns a method of preparation of the nanofiber structure with immobilized organic agents, while silica nanofibers are prepared by electrostatic spinning from the initial sol synthesized by a sol-gel method from tetraalkoxysilane, heat-treated and their the surface modified by a solution of aminoalkylalkoxysilane and organic agents is then immobilized on the modified surface of the nanofibers.

NANOFIBER STRUCTURE WITH IMMOBILIZED ORGANIC SUBSTANCE AND THE METHOD OF ITS PREPARATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a 35 U.S.C. §§371 national phase conversion of PCT/CZ2012/000128, filed Dec. 10, 2012, which claims priority to Czech Republic Patent Application No. PV 2012-549, filed Aug. 14, 2012, the contents of which are incorporated herein by reference. The PCT International Application was published in the English language.

TECHNOLOGY AREA

[0002] The invention concerns a nanofiber structure with immobilized organic substance.

[0003] The invention also concerns a method of preparation of the nanofiber structure with immobilized organic substance.

EXISTING STATE OF TECHNOLOGY

[0004] In many medicine and biotechnology applications functional organic substance (medicines, enzymes etc.) is applied by being placed into the respective environment where they should operate, i.e. into wounds, or biochemical reactors etc. Sometimes the functional organic substance need to be significantly overdosed because there are significant losses of the substance due to dispersion or washing away (typically washing of antibiotics from the wound etc.) or it is necessary to administer the substance frequently. This, however, may cause permeation of the substance into other parts of the organism and undesired side effects or, on the contrary, insufficient efficiency of the applied substance.

[0005] In biochemical applications the employed organic substance (mostly enzymes) usually cannot be recovered from the application area, e.g. for further use, and a new quantity of the functional organic substance needs to be added into the next production batch. This, however, significantly increases costs and the reaction product contains free enzyme which is not desirable from the viewpoint of further product use.

[0006] These problems may be eliminated or limited by immobilization of organic substance on suitable substrates, i.e. ideally by permanent or at least as long as possible lasting attachment of organic substance on suitable carriers. The preconditions of this approach include, apart from a sufficiently stable immobilization of the organic substance on the substrate, keeping of the efficiency and function of the substance and also a sufficient quantity of the immobilized substance.

[0007] The quantity of the immobilized substance depends on the number of suitable bonding places for immobilization and on the specific surface of the substrate. From this point of view nanofibers are a particularly suitable substrate because their specific surface is in units to tens of m^2/g while they keep suitable mechanical properties; those properties make it possible to form shapeable nanofiber layers that may be easily placed into a wound or into a holder in a biochemical reactor and after completion of the application they may be removed.

[0008] The patents CZ 294274 or WO 2005024101 describe a method of nanofiber preparation by electrostatic

spinning. However, the patents CZ 294274 or WO 2005024101, do not specify in detail polymer solutions for preparation of the nanofibers.

[0009] In the patent WO2009018104 methyltrimethoxysilane is used as a precursor to prepare silica nanofibers. Without heat treatment according to WO2009018104 or if heat-treated at low temperatures according to WO2009018104, the nanofibers will have hydrophobic properties as a result of presence of methyl groups on the surface and the number of Si—OH groups on the surface for its subsequent potential modification with aminoalkylalkoxysilane will be low. For this reason the procedure under WO 2009018104 is not suitable for preparation of initial silica nanofibers for the subsequent stages of modification and immobilization of organic substance.

[0010] The patents JP20040041335, JP20040161234 and JP20040243580 describe preparation of an organic-inorganic nanofiber composite made of a regular framework of polyethylene imide fibers with layers of silicon dioxide applied with a sol-gel method. The resulting composite material is intended to trap, or to increase concentrations of, various substances in the prepared structure, however, only as a filter, i.e. in gaps between individual nanofibers, or by simple adsorption of the desired particles in the bulk nanofiber composite.

[0011] The packaging paper under the patent KR20090058155 is made of nanofibers obtained by electrostatic spinning of a biodegradable organic polymer with addition of sol of silicon dioxide and silver nitrate. The resulting product has antiseptic and antibacterial properties but it is not suitable for immobilization of organic substance.

[0012] The patent KR20100058372 describes preparation of a catalyst from mesoporous nanofibers of silicon dioxide prepared by growing from a gaseous phase and subsequent introduction of a catalyst with silane on the surface and into the pores of the nanofibers prepared in this manner. The resulting product is described as a catalyst of various organic reactions and it is not used for immobilization of organic substance.

[0013] The drawback of the existing state of technology consists in the absence of a sufficiently biochemically stable structure capable of sufficiently high, efficient and, in terms of time, stable immobilization of organic substance.

[0014] The objective of this invention is to eliminate, or at least to minimize, disadvantages of the current state of technology.

PRINCIPLE OF THE INVENTION

[0015] The objective of the invention is achieved by a nanofiber structure with immobilized organic substance, the principle of which consists in the fact that silica nanofibers have their surface modified by a reaction with aminoalkylalkoxysilane and subsequently organic substance is immobilized on their surface with peptide or hydrogen bonds.

[0016] Silica nanofibers are suitable particularly thanks to their high stability in biochemical reactions and thanks to their dissolving ability in body fluids. The dissolving rate of silica nanofibers is controlled by the temperature of thermal processing of the silica nanofibers and therefore the nanofibers may be removed from the wound along with the immobilized substance after a previously specified time and the residues of silica nanofibers potentially released in the place of application, in a wound or bioreactor, e.g. by breaking etc., are then dissolved in body fluids or in the bioreactor suffi-

ciently quickly without any negative side effects. Silica nanofibers have another advantage that their surface with high numbers of Si—OH groups can be easily modified with reactions in which Si—OH groups are linked via covalent bonds with aminoalkylalkoxysilanes whose amino groups subsequently enable formation of relatively strong peptide bonds or slightly weaker hydrogen bonds with the immobilized organic substance.

[0017] The principle of the method of preparation of the nanofiber structure with immobilized organic substance consist in preparation of an initial sol from tetraalkoxysilane using a sol-gel method and the sol is then exposed to electrostatic spinning; the resulting nanofiber structure is heat-treated and its surface is treated with aminoalkylalkoxysilane; the surface of the nanofiber structure is then exposed to a solution organic substance and the organic substance is immobilized by means of peptide or hydrogen bonds on the surface of the nanofiber structure.

[0018] The basis of this method is to create a nanofiber structure, e.g. in form of silica nanofibers made by electrostatic spinning from a sol prepared by a sol-gel method from tetraalkoxysilane using acidic catalysis and without additional organic polymers. In order to obtain a sol with suitable properties for electrostatic spinning it is necessary to observe the molar ratio of water to tetraalkoxysilane $k=[H_2O]/[tetraalkoxysilane]$ in the range $k=1.6$ to 3 , the molar ratio of acid to tetraalkoxysilane $m=[HA]/[tetraalkoxysilane]$ in the range $m=0.001$ to 1 and the sol shall be concentrated before spinning by evaporation of the solvent to achieve the SiO_2 concentration in the sol in the range 28 to 44 wt. %.

[0019] This method can be used to prepare silica nanofibers with the average diameter in the range 100 to 1000 nm (depending on the conditions of preparation of the initial sol and the conditions of electrostatic spinning) to form a nanofiber structure (layer) that can be directly used for subsequent operations, without the necessity to remove any additives by heat treatment at high temperatures. Specific surfaces of nanofiber structures (layers) prepared in this manner range from units to tens of m^2/g and thus they ensure large surfaces for immobilization of organic substance even if the nanofiber layer is thin.

[0020] The resulting properties of the nanofiber structure, e.g. the number of active Si—OH groups for subsequent bonds with aminoalkylalkoxysilane and chemical durability against water and body fluids, are fundamentally affected by a sufficient heat treatment of the nanofiber layer before immobilization of organic substance. The morphology of nanofibers practically does not change up to the temperature around $850^\circ C.$, when it transforms into silica glass (except a slight reduction of their diameters as a result of their thickened structure at high temperatures). During the heat treatment silica nanofibers gradually reduce their chemical solubility and the number of active Si—OH groups for subsequent bonds with aminoalkylalkoxysilane. Sufficient chemical solubility of silica nanofibers in body fluids is necessary because the size of nanofiber fragments released during manipulation with the nanofiber structure and their accidental inhalation is in the area of documented carcinogenicity in case of their long-term local presence (more than 40 days). During dynamic and static tests of silica nanofibers dissolving (average diameter around 180 nm) in simulated lung fluid the dissolving rate of nanofibers was strongly dependent on the temperature of thermal processing of the nanofibers. In case of heat treatment at low temperatures ($180^\circ C./2$ hours) the

nanofibers dissolved within 7 days and they can be considered safe for manipulation and in case of accidental inhalation. However, in case of heat treatment at higher temperatures the nanofibers gradually became less soluble and the nanofibers heat-treated in this manner shall be viewed as potentially carcinogenic.

[0021] Most of medically or biochemically active organic substance contain carboxyl (—COON) or at least hydroxyl (—COH) groups. However, to ensure a sufficient level and reliability of immobilization of such organic substance on the surface of silica nanofibers it is necessary to modify the surface of the nanofibers so that there are firmly bound amino groups on it to form peptide bonds with carboxyl groups or hydrogen bonds with hydroxyl groups. In this manner the organic substance is sufficiently immobilized on the surface of nanofibers. The surface of nanofibers is therefore modified by a solution of aminoalkylalkoxysilane which forms covalent bonds, by polycondensation via alkoxy groups, with surface Si—OH groups of silica nanofibers and with its free aminoalkyl group provides a primary functional amino group for the formation of peptide or hydrogen bonds with organic substance. In a suitable solvent or environment (water, alcohol or other organic solution) the formation of bonds between amino groups and organic substance is spontaneous. The immobilization of organic substance on the surface of silica nanofibers with the surface modified according to this invention is sufficiently strong to ensure that during the subsequent application of the nanofiber structure with immobilized organic substance, e.g. in presence of water or body fluids, like in open wounds etc., the immobilized organic substance operates but it is not released from the nanofiber structure or is released only in small quantities, i.e. only in minimum quantities. This ensures a long-term, contact and high concentration of organic substance at a place of their desired application, without being washed away and without excessive release from the place of application.

EXAMPLES OF EXECUTION OF THE INVENTION

[0022] The invention will be described on examples of the procedures to prepare nanofiber structure in form of a layer of silica nanofibers with modified surface and immobilized organic substance on the surface. In the following text the invention is documented with specific examples which, however, do not document all possibilities of the invention whose application and use are obvious to an average expert from this text without any additional inventing efforts.

Example 1

[0023] The initial sol for preparation of silica nanofibers was prepared with a modified sol-gel method. 400 ml of tetraethoxysilane were dissolved in 330 ml isopropyl alcohol and water and HCl were added to achieve the molar ratio $k=[H_2O]/[tetraalkoxysilane]=2.3$ and the molar ratio $m=[HCl]/[tetraalkoxysilane]=0.01$. After completion of hydrolytic and polycondensation reactions the sol was concentrated by evaporation of the solvent to 36 wt. % of SiO_2 .

[0024] The prepared sol was used for electrostatic spinning at the voltage of 50 kV and the distance of 15 cm. The average size of the prepared nanofibers was 180 nm. The resulting nanofiber structure in form of a layer of nanofibers was heat-treated at $180^\circ C.$ for 2 hours in a drying kiln and the surface of silica nanofibers was modified by immersion into a 2%

solution of 3-aminopropyltriethoxysilane in anhydrous ethanol for 1 hour at the laboratory temperature. The modified nanofiber structure was washed three times with anhydrous ethanol and submerged into 2% solution of tetracycline or penicillin in anhydrous ethanol for 2 hours at the laboratory temperature. Finally, the nanofiber structure with the immobilized antibiotic (organic substance) was flushed twice with anhydrous ethanol and left to dry in a desiccator.

[0025] The function of the nanofiber structure with the immobilized antibiotic was verified by antibacterial tests on a selected group of pathogenic bacterial strains that may cause problems particularly in dermatology. They included bacterial strains *Staphylococcus aureus*, MRSA, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Samples of the nanofiber structure with the immobilized antibiotic were placed into a center of a Petri dish with respective bacterial inoculum of a selected bacterial strain. The samples were incubated in a thermostat for 24 hours at 37° C. Further, the size of the so-called halo zones was evaluated (i.e. zones around the nanofiber structure with immobilized antibiotics).

[0026] Results of the antibacterial tests were excellent—the sizes of tested halo zones were evaluated as 100% inhibition ability of the antibiotics against the selected bacterial pathogenic strains. This has confirmed that both the antibiotics were immobilized on the nanofiber structure of silica nanofibers with modified surface hereunder, without losing their function.

Example 2

[0027] The initial sol for preparation of silica nanofibers was prepared by a modified sol-gel method. 400 ml of tetraethoxysilane were dissolved in 330 ml isopropyl alcohol and water and HCl were added to achieve the molar ratio $k=[\text{H}_2\text{O}]/[\text{tetraalkoxysilane}]=2.0$ and the molar ratio $m=[\text{HCl}]/[\text{tetraalkoxysilane}]=0.01$. After completion of hydrolytic and polycondensation reactions the sol was concentrated by evaporation of the solvent to 40 wt. % of SiO_2 .

[0028] The prepared sol was used for electrostatic spinning at the voltage of 50 kV and the distance of 15 cm. The average size of the prepared nanofibers was 580 nm. The resulting nanofiber structure was in form of a layer of nanofibers was heat-treated at 180° C. for 2 hours in a drying kiln and the surface of nanofibers was subsequently modified with a 2% solution of 3-aminopropyltrimethoxysilane in distilled water for 1 hour at the laboratory temperature. The nanofiber structure with the modified surface was washed three times with distilled water and once with a solution of phosphate buffer (pH=7.2) and then it was submerged into a 2% solution of esterase or lipase enzyme in phosphate buffer for 10 minutes at the laboratory temperature. Finally, the nanofiber layer with the immobilized enzyme was flushed two times with phosphate buffer and left to dry in the laboratory environment.

[0029] The immobilization of the enzymes on the nanofiber structure was verified with a histochemical azocoupling reaction of alpha-naphthyl acetate and chromogenic dye with the enzyme. The solution of alpha-naphthyl acetate and Fast Blue BB dye in phosphate buffer in combination with the enzyme formed colored deposits of immobilized enzyme that were visible in a microscope and demonstrated its presence. The tests were performed as comparative against a nanofiber

structure made of silica nanofibers on which no enzyme was immobilized hereunder, while no deposits as described above were found on the control nanofiber structure without the enzymes.

1. A nanofibrous structure with immobilized organic substance composed of pure silica nanofibers made by electrostatic spinning, the nanofibers have controlled dissolution in body fluids or in bioreactor and have surface modified by aminoalkylalkoxysilane with subsequently immobilized organic agent substances.

2. The method of production of nanofibrous structure with immobilized organic substance, wherein, from the initial sol synthesized by the sol-gel method from tetraalkoxysilane by means of electrostatic spinning the pure silica nanofibers are created, which are subsequently heat treated for controlled dissolution in body fluids or in bioreactor and then surface of nanofibers is modified by means of solution of aminoalkylalkoxysilane, after which to such modified surface of nanofibers the organic substances are immobilized.

3. The method according to the claim 2, wherein the initial sol is prepared by the sol-gel method from solution of tetraalkoxysilane in alcohol with addition of water upon an acid catalysis by controlled hydrolysis and polycondensation and by thickening through distilling-off the solvent to a viscosity necessary for electrostatic spinning.

4. The method according to the claim 3, wherein the alcohol is ethanol or isopropylalcohol, an acid catalysis of hydrolysis and polycondensation of tetraalkoxysilane is ensured by addition of inorganic or organic acid, the initial molecular ratio of water to tetraalkoxysilane $k=[\text{H}_2\text{O}]/[\text{tetraalkoxysilane}]$ is within values $k=1.6$ to 3, molecular ratio of an acid to tetraalkoxysilane $m=[\text{HA}]/[\text{tetraalkoxysilane}]$ is within values $m=0.001$ to 1 and the sol before spinning is thickened by evaporation of the solvent to the content of SiO_2 in the sol to be within values 28 to 44% by weight.

5. The method according to the claim 4, wherein the tetraalkoxysilane is tetraethoxysilane and the acid is hydrochloric acid or nitric acid.

6. The method according to the claim 2, wherein through the electrostatic spinning purely silica nanofibers having mean diameter 100 to 1000 nm are prepared, which are then heat treated within temperature 30 to 900° C. for a period of 10 minutes to 10 hours.

7. The method according to the claim 6, wherein the pure silica nanofibers are heat treated at a temperature within 150° C. to 250° C. for a period 1 to 3 hours.

8. The method according to the claim 2, wherein the solution of aminoalkylalkoxysilane is in water, alcohol or other organic solvent and it has concentration of 0.1 to 10% by weight.

9. The method according to the claim 8, wherein the aminoalkylalkoxysilane is 3-aminopropyltriethoxysilane or 3-aminopropyltrimethoxysilane.

10. The method according to the claim 2, wherein the organic agent substance is immobilized to the modified surface of nanofibers in the liquid environment in a period of 30 seconds to 48 hours.

11. The method according to the claim 10, wherein the liquid environment is water, aqueous solution of biochemical buffer, ethanol, isopropylalcohol or acetone and immobilization time varies from 3 minutes to 24 hours.

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