(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 August 2017 (03.08.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/129153 A1

(51) International Patent Classification:
A01N 25/10 (2006.01) *B05D 1/18* (2006.01)
A01N 59/16 (2006.01) *B82Y 30/00* (2011.01)
A01P 1/00 (2006.01) *B82Y 40/00* (2011.01)

(21) International Application Number:
PCT/CZ2017/050002

(22) International Filing Date:
25 January 2017 (25.01.2017)

(25) Filing Language: Czech

(26) Publication Language: English

(30) Priority Data:
PV 2016-38 27 January 2016 (27.01.2016) CZ

(71) Applicant: **REGIONALNI CENTRUM POKROCILYCH TECHNOLOGII A MATERIALU, UNIVERZITA PALACKEHO V OLOMOUCI** [CZ/CZ]; Slechtitelu 11, 78371 Olomouc (CZ).

(72) Inventors: **SOUKUPOVA, Jana**; Jarmily Glazarove 21, 77900 Olomouc (CZ). **ZBORIL, Radek**; Jarmily Glazarove 354/9D, 77900 Olomouc (CZ).

(74) Agent: **HARTVICHOVA, Katerina**; INVENTIA s.r.o., Na Belidle 3, 15000 Praha 5 (CZ).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2017/129153 A1

(54) Title: POLYMER SUBSTRATE WITH IMMOBILIZED SILVER NANOPARTICLES AND METHOD OF PREPARATION THEREOF

(57) Abstract: The invention provides a method of preparation of a polymer substrate with immobilized silver nanoparticles, wherein the substrate is prepared via electro spinning of a mixture of water non-soluble polymer and a polymer linker containing Ag-reducing functional groups containing nitrogen atom having a free electron pair, and consequently, the substrate is subjected to aqueous solution of silver ions resulting in formation of silver nanoparticles covalently immobilised to the substrate via nitrogen atom of the polymer linker. Further, an antimicrobial material is described, which contains the substrate from water non-soluble polymer and polymer linker containing functional groups containing nitrogen atom with a free electron pair. Silver nanoparticles are covalently immobilized to these functional groups of.

Polymer Substrate with Immobilized Silver Nanoparticles and Method of Preparation Thereof

Filed of Art

The present invention relates to a method of preparation of a polymer composite substrate with immobilized silver nanoparticles and to the substrate obtainable using the method.

Background Art

Nowadays, there are several approaches to tailored immobilization of silver nanoparticles on various substrates (AgNPs) with the aim to provide antimicrobial treatment of the material or to provide antifouling protection (prolongation of lifetime of the filtration/fibrous structures). These approaches, however, do not solve the problem of potential nanoparticle release, potentially release of the silver nanoparticles with their linkers. The Czech patent CZ303502 describes the approach to immobilization of silver nanoparticles (AgNPs) via covalent bonds between the silver and the linker but it does not eliminate possible release of the whole composite, i.e. Ag-linker, from the substrate under mechanical stress or under high pressure of the flow and flow rate of the media – in the case of the filtration materials.

The above-described problem is solved by the present invention, which provides for the immobilization of silver nanoparticles onto the substrate in such a way that effectively prevents possible release of the silver nanoparticles/silver nanoparticles with a polymer linker from the composite substrate. This immobilization process represents rather simplified method as the functionalization of the surfaces can proceed under significantly lower temperatures. The temperature represents a crucial parameter especially from some substrates.

Disclosure of the Invention

The present invention introduces a method of preparation of a polymer substrate with immobilized silver nanoparticles, wherein the substrate is prepared from a mixture of water

non-soluble polymer and a polymer linker containing Ag-reducing functional groups (i.e. functional groups that are able to reduce Ag^+ to Ag^0) containing nitrogen atom with a free electron pair. Consequently, the substrate is functionalized with covalently bonded silver nanoparticles, which are generated from an aqueous solution of silver ions. The generated silver nanoparticles are covalently bonded to the substrate via the nitrogen atom and/or sulphur atom of the polymer linker.

Water non-soluble polymer is preferably a synthetic polymer, more preferably selected from a group comprising polyurethanes, polylactide, polyglycolides, poly(lactide-co-glycolide), polyethylene, polypropylene, polyamide, saccharides, as for example cellulose or chitosan.

Ag-reducing functional groups comprise all groups containing nitrogen and at the same time having a free electron pair, i.e. for instance amino-, imino-, imido- functional group.

The polymer linker has preferably molecular weight from 300 $g.mol^{-1}$ to 1,000,000 $g.mol^{-1}$ and has either linear or branched structure. A preferable polymer linker is e.g. polyethylenimine.

The substrate is nanofibrous and is prepared from a water non-soluble polymer and a polymer linker using electrospinning method. It has been proved that if the substrate is prepared from a mixture of water non-soluble polymer and polymer linker, it is sufficient to prevent the undesired release of the composite of Ag-linker. The release of Ag-linker composite represented the disadvantage in CZ 303502.

Preferably, the polymer linker is involved in the substrate in the concentration from 0.0001 wt. % up to 5 wt.%. The concentration is related to the overall mass of the water non-soluble polymer.

Silver ions might be in a form of a compound containing silver ions, which can be a water soluble silver salt, as e.g. $AgNO_3$, $AgClO_4$, AgF, or a water soluble complex compound of silver ions, as e.g. diamminesilver complex.

Aqueous solution containing ionic silver reacts with the substrate, preferably in the concentration range of silver ions solution from 0.0001 $mol \cdot L^{-1}$ up to 5 $mol \cdot L^{-1}$; preferably at the temperature of from 5 °C to 95 °C, ideally at room temperature, preferably for the period from 1 s up to 60 minutes.

The resulting composite material exhibit the required antimicrobial properties thanks to the covalently bonded silver nanoparticles, which are even firmly anchored through the polymer linker, which itself is anchored into the structure of the substrate. In contrast to the method, when the silver nanoparticles are anchored into the substrate, in the method according to the present invention the overall surface of the silver nanoparticles is available for the antimicrobial action, and the silver nanoparticles do not release from the surface under mechanical stress, which represents a key parameter of such a composite for the use e.g. in the filtration/water treatment technologies or in medicinal applications.

The subject of the present invention is further an antimicrobial material containing silver nanoparticles, which structure is based on a substrate formed by a water non-soluble polymer and polymer linker containing a nitrogen atom and/or a sulphur atom onto which silver nanoparticles are covalently bonded. Preferably, the antimicrobial material can be prepared using the method according to the present invention.

Brief Description of Figures

Fig. 1 shows the SEM image of the nanofibers prepared according to the example 1.
Fig. 2 shows the SEM image of the nanofibers prepared according to the example 2.
Fig. 3 shows the SEM image of the nanofibers prepared according to the example 3.

Examples of the Invention Embodiments

Example 1: Polyurethane (PU) fibres with immobilized silver nanoparticles (on all surfaces)

Nanofibrous structures of polyurethane (PU), i.e. fibres with a diameter in the range from tens up to hundreds of nanometers, are prepared by electrospinning from the mixture of polyurethane dissolved in dimethylformamide (DMF). The weight percentage of PU dissolved in DMF is 4%. After homogenization of the DMF:PU mixture, the branched polyethylenimine (i.e. hyperbranched polymer linker containing nitrogen atoms with free electron pairs, which can be used for the reduction stabilization of subsequently generated and immobilized silver nanoparticles) was added. The molecular weight of the polyethylenimine was 25,000 $g.mol^{-1}$ (in the concentration of 1 wt.% to the PU mass). The homogenization of the mixture DMF:PU:PEI is followed by electrospinning of this mixture. The fibres are collected on a rotation collector as a compact layer of the mesh structured fibres (i.e. fibres without orientation). This structure contains fibres with immobilized polymer linker in its structure. Thanks to the immobilized linker the fibres can be preferably used for tailored deposition of silver nanoparticles when the fibres are immersed into the precursor of the silver nanoparticles solution, i.e. into silver nitrate with the concentration of $8 \cdot 10^{-4}$ $mol.L^{-1}$. The mesh structure is taken out of the solution immediately and the structure is washed with water in order to get rid of the residues of the precursor from all the mesh cavities. The whole process is carried out under room temperature. There is a strong coordination-covalent interaction between the heteroatom and silver, and the linker itself is firmly anchored in the substrate structure. Based on the above-mentioned reasons the release of the silver nanoparticles or silver nanoparticles with the linker from the composite material is prevented.

The amount of silver in the prepared sample of PU fibres with immobilized AgNPs was consequently quantified with AAS. Comparable sample was placed into water and onto a shaker and let to be mechanically stressed. After 24 hours, the amount of silver on the composite material was quantified with AAS. The amount of silver on the tested (i.e. first sample) and stressed (i.e. the sample mechanically stressed for 24 hours in water on a shaker) sample was almost identical (note: the difference was 0.1 wt% of silver, which represents a statistical error can correspond with the released Ag^+ ions released into the aqueous environment; the antimicrobial effect is probably based on the release of the Ag^+ from the silver nanoparticle surface).

The structure of the PU fibres with immobilized molecules of hyperbranched polyethylenimine and consequently immobilized silver nanoparticles on the substrate

surface proved to exhibit antimicrobial efficiency against model organisms of strains of *Staphylococcus* and *Escherichia* (tested according to the norm JIS1902/ISO20743 for textiles). The tested samples were 5x5 cm in size and the concentration of the inoculum was 10^5 CFU/mL. The samples functionalized with silver nanoparticles proved to supress the growth of bacterial colonies completely in contrast to the blank samples (the PU fibres without functionalization).

Example 2: Fibres of poly(lactide-co-glicolide) (PLGA) with immobilized silver nanoparticles on all surfaces.

Nanofibrous structures of poly(lactide-co-glycolide) (PLGA), i.e. fibres of PLGA with the average diameter of hundreds of nanometers are prepared via electrospinning from the mixture of PLGA in chloroform ($CHCl_3$, 95%) with the addition of dimethylformamide (DMF; 5%). PLGA with the molecular weight of 50,000 – 75,000 $g.mol^{-1}$ is dissolved in the mixture of chloroform and DMF in the weight ratio of 30%. After homogenization of the mixture of PLGA:DMF:$CHCl_3$ on a shaker, hyperbranched polyethylenimine (PEI) with molecular weight 25,000 $g.mol^{-1}$ was added in the concentration of 0.1 wt.% (in the relation to the PLGA mass). (Note: The hyperbranched polymer linker contains numerous nitrogen atoms with free electron pair, which is used for the reduction, stabilization and immobilization of the generated silver particles.) The resulting mixture is again homogenized on a shaker. Subsequently, oriented fibres are prepared via electrospinning and collected on a rotation collector. This way prepared fibres, containing polymer linker in its structure for targeted deposition of silver nanoparticles, are consequently immersed into the solution of silver nanoparticle precursor, i.e. into silver nitrate with the concentration equal to $5 \cdot 10^{-3}$ $mol.L^{-1}$, for the period of 3 minutes. Then they are taken out and washed with distilled water in order to eliminate the chemical residues, especially traces of silver nitrate as the nanoparticle precursor. There is a strong coordination-covalent interaction between the heteroatoms and silver. The linker itself is firmly anchored in the structure of the fibre, which prevents release of either silver nanoparticles or silver nanoparticles with the polymer linker.

Example 3: Nanofibres of polylactide-co-glycolide (PLGA) with immobilized silver nanoparticles on all fibre surfaces.

Nanofibrous structures of polylactide-co-glykolide (PLGA), i.e. fibres with the diameter of tents of nanometers are prepared via electrospinning from the mixture of PLGA dissolved in tetrahydrofuran (THF, 50%) and chloroform ($CHCl_3$, 50%). PLGA (with the molecular weight of 50,000 – 75,000 $g.mol^{-1}$) is dissolved in the mixture of chloroform and THF in the weight ratio of 30%. After homogenization of the mixture of PLGA:THF:$CHCl_3$ on a shaker, hypebranched polymer linker – polyethylenimine is added to the mixture (the molecular weight of polyethylenimine is 750,000 $g.mol^{-1}$; note: the hyperbranched polymer linker contains sufficient number of nitrogen atoms with free electron pair, which is used in the process of reduction, stabilization and immobilization of silver nanoparticles)) in the concentration of 0.1 wt.% (in the relation to the PLGA mass). Subsequently, the fibres were prepared via electrospinning. The fibres were collected on a rotation collector without any orientation – in a form called "mesh". This way prepared fibres, involving polymer linker in its structure for targeted deposition of silver nanoparticles, are consequently immersed into the solution of silver nanoparticle precursor, i.e. into silver nitrate with the concentration equal to $1 \cdot 10^{-2}$ $mol.L^{-1}$, for the period of 3 minutes. Then they are taken out and properly washed with distilled water in order to eliminate the chemical residues, especially the any traces of the silver nitrate as the precursor. There is a strong coordination-covalent interaction between the heteroatoms in polymer linker structure and silver. The linker itself is firmly anchored in the structure of the fibre, which prevents release of either silver nanoparticles or silver nanoparticles with the polymer linker.

CLAIMS

1. A method of preparation of a polymer substrate with immobilized silver nanoparticles, characterized in that the substrate is prepared via electrospinning of a mixture of water non-soluble polymer and a polymer linker containing Ag-reducing functional groups containing nitrogen atom having a free electron pair, and consequently, the substrate is subjected to aqueous solution of silver ions resulting in formation of silver nanoparticles covalently immobilised to the substrate via nitrogen atom of the polymer linker.

2. The method according the claim 1, characterized in that the water non-soluble polymer is a synthetic polymer, preferably selected from a group comprising polyurethanes, polylactides, polyglycolides, poly(lactide-co-glycolide), polyethylene, polypropylene, polyamide, saccharide, as e.g. cellulose or chitosan.

3. The method according to the claim 1 or 2, characterized in that the Ag-reducing functional groups are selected from the group comprising amino-, imino-, imido groups.

4. The method according to any one of the preceding claims, characterized in that the polymer linker is polyethylenimine.

5. The method according to any one of the preceding claims, characterized in that the polymer linker is contained in the substrate in the concentration range from 0.0001 wt. % to 5 wt. %, related to the overall mass of the water non-soluble polymer.

6. The method according to any one of the preceding claims, characterized in that the silver ions are in a form of a water soluble silver salt or a water soluble complex of silver ions.

7. The method according to any one of the preceding claims, characterized in that the application of the aqueous solution of silver ions on the substrate is performed at the concentration of the silver ions in the range of from 0.0001 $mol \cdot L^{-1}$ to 5 $mol \cdot L^{-1}$;

preferably at the temperature of the media from 5 °C to 95 °C, preferably for the time period from 1 s to 60 minutes.

8. Antimicrobial material with the content of silver nanoparticles, characterized in that it contains nanofibrous substrate from a mixture of water non-soluble polymer and a polymer linker containing functional groups comprising nitrogen atoms with free electron pairs, wherein silver nanoparticles are covalently immobilized to those functional groups.

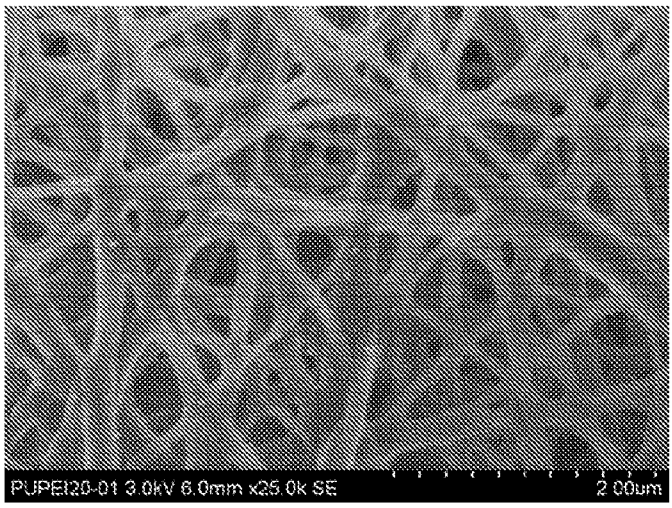
PUPEI20-01 3.0kV 6.0mm x25.0k SE
2.00um

Fig. 1

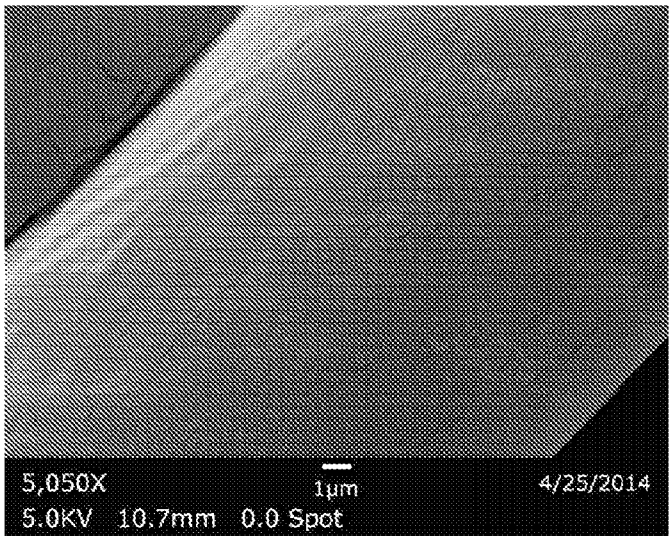
5,050X
1µm
4/25/2014
5.0KV 10.7mm 0.0 Spot

Fig. 2

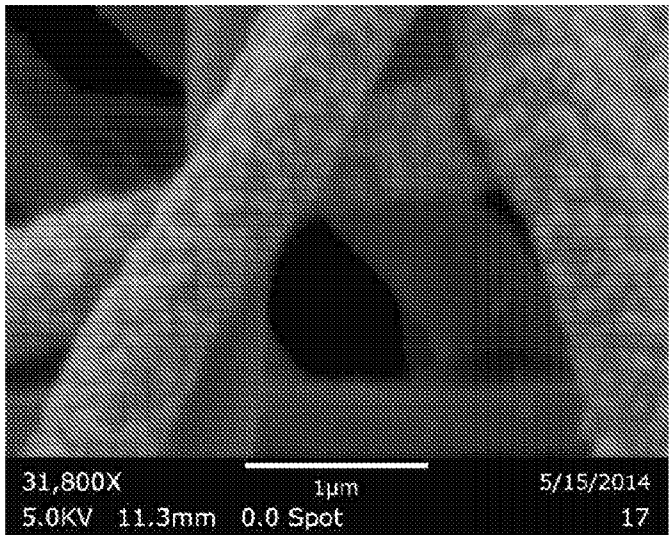

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2017/050002

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N25/10 A01N59/16 A01P1/00 B05D1/18 B82Y30/00
B82Y40/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N B05D B82Y

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/116408 A1 (CALIFORNIA INST OF TECHN [US]; KOREA ADVANCED INST SCI & TECH [KR]) 8 August 2013 (2013-08-08)	1-8
Y	examples 20, 37	1-8
Y	WO 2013/029574 A1 (UNIVERZITA PALACKEHO V OLOMOUCI [CZ]; ZBORIL RADEK [CZ]; SOUKUPOVA JAN) 7 March 2013 (2013-03-07) cited in the application page 7, lines 8-18	1-8
Y	KR 2010 0026083 A (UNIV KOREA RES & BUS FOUND [KR]) 10 March 2010 (2010-03-10) paragraphs [0024], [0034], [0041] - [0044]	1-8

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 May 2017	23/05/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Davies, Maxwell

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2017/050002

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KR 2007 0078177 A (INHA IND PARTNERSHIP INST [KR]) 31 July 2007 (2007-07-31) claim 1	1-8
Y	US 2008/217807 A1 (LEE BONG DAE [KR] ET AL) 11 September 2008 (2008-09-11) paragraphs [0050], [0053] claims 1-11	1-8

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CZ2017/050002

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013116408 A1	08-08-2013	US 2013213881 A1	22-08-2013
		US 2016243504 A1	25-08-2016
		WO 2013116408 A1	08-08-2013
WO 2013029574 A1	07-03-2013	CZ 303502 B6	24-10-2012
		EP 2701515 A1	05-03-2014
		US 2015290677 A1	15-10-2015
		WO 2013029574 A1	07-03-2013
KR 20100026083 A	10-03-2010	NONE	
KR 20070078177 A	31-07-2007	NONE	
US 2008217807 A1	11-09-2008	JP 2008095266 A	24-04-2008
		US 2008217807 A1	11-09-2008