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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
— *of inventorship (Rule 4.17(iv))*

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 2018/082721 A1

(54) Title: METHOD FOR PRODUCING A NANOFIBER LAYER AND A TEXTILE COMPOSITE COMPRISING THE NANOFIBER LAYER

(57) Abstract: A method for producing a nanofiber layer based on chitosan in a solution with polyethylene oxide (PEO), designed for a textile composite for wound dressing, consists of the following steps: - mixing the chitosan monomer with a methacrylate-containing agent in an aqueous medium, - subjecting the mixture to filtration, washing it with H_20 and drying it without access to light, - crushing the resulting product into powder, soluble solid phase modified chitosan, - subsequently preparing a spinning mixture by dissolving the modified chitosan and polyethylene oxide (PEO) in an aqueous acetic acid solution, - applying the spinning solution prepared in this way on a suitably selected substrate, - crosslinking the nanofiber layer material.

Method for producing a nanofiber layer and a textile composite comprising the nanofiber layer

Technical Field

The invention relates to a method for producing a nanofiber layer based on chitosan in a solution with polyethylene oxide (PEO), designed as a textile composite for wound dressing, as well as a primary wound cover containing a nanofiber layer, material composition of the nanofiber layer, and the connection of the nanofiber layer with an absorbent or mechanical carrier.

Background Art

At present, experts are engaged in the production of nanofibers from biocompatible materials, whose properties imitate actual tissues and which the body is then able to absorb to a greater or lesser extent. There are various ways of using the nanofiber layer and its saturation with various therapeutic agents, as well as various ways of linking this layer with the carrier.

Document TW 200740472 describes the method of manufacturing a wound dressing fabric comprising a nanofiber layer formed by electrostatic spinning. The acidic spinning solution contains collagen, chitosan and polyethylene oxide (PEO). The paper does not mention the steps that would lead to the stabilization of the nanofiber layer in a humid environment.

KR 20150125881 also describes a process for making a nonwoven fabric from nanofibers. The spinning solution contains water-soluble chitosan and PEO. The procedure does not include any steps that would lead to the structural stabilization of the nanofiber layer.

US 20130150763 relates to a wound dressing fabric composed of three nanofiber layers. The first, external hydrophobic layer consisting of nanofibers from biocompatible polymers has a supportive function. The second, middle, hydrophilic layer crosslinked by genipin serves as a reservoir of biologically active substances,

the spinning solution at a chitosan ratio of 0.5 to 10 wt%. The crosslinking of chitosan nanofibers by genipin inhibits the initial release of herbal extracts and prolongs their release time. The third, hydrophilic layer comprising non-crosslinked chitosan provides the dressing's contact with the wound. Various additives are added to these layers in the exemplary versions, which influence the speed of release for herbal extracts.

A disadvantage of this solution is the cost associated with the use of genipin as a crosslinking agent.

The objective of the present invention is to provide a method for producing a stabilized nanofiber layer based on chitosan without the use of expensive chemical crosslinkers, which in addition allows crosslinking as required at any time after the production of the nanofiber layer.

Disclosure of the invention

The objective of the present invention is to provide a method for producing a nanofiber layer based on chitosan in a solution with polyethylene oxide (PEO) designed for a textile composite for wound dressing. The method comprises the following steps:

- mixing the chitosan monomer with a methacrylate-containing agent in an aqueous medium,
- subjecting the mixture to filtration, washing it with H_2O and drying it without access to light,
- crushing into powder the resulting product, which is soluble solid phase modified chitosan,
- subsequently preparing a spinning mixture by dissolving the modified chitosan and polyethylene oxide (PEO) in an aqueous acetic acid solution,
- applying the spinning solution prepared in this way on a suitably selected substrate,
- crosslinking the nanofiber layer material.

The most advantageous version of the method comprises the following steps:

- mixing a chitosan monomer with -NH_2 group at 80% deacetylation dissolved in an aqueous environment at a ratio of 5 g chitosan to 10 ml water with glycidyl methacrylate (GMA) at a ratio of 2 mol chitosan to 1 mol GMA, and mixing the mixture for 24 hrs.,
- subjecting the mixture to filtration, washing it by H_2O and drying it for two days without access to light,
- crushing the resulting product into powder, which is solid phase modified chitosan soluble in an acidic aqueous medium,
- subsequently preparing a spinning mixture by dissolving the modified chitosan and PEO in an aqueous acetic acid solution at the volume ratio of water to acetic acid 2 : 1 in such a way that the resultant solution contains 2 wt% to 10 wt% chitosan and 0.1wt% to 0.3 wt% PEO,
- applying the spinning solution prepared in this way on a suitably selected substrate,
- crosslinking the nanofiber layer material.

Crosslinking can be performed by exposing the nanofiber layer to UV radiation.

In another version of the invention, the method can comprise the following steps:
- adding gelatine to the modified chitosan and PEO solution in such a way that the resultant solution contains 4 wt% to 9 wt% chitosan, 0.1 wt% to 0.3 wt% PEO and 0.5 wt% to 5.0 wt% gelatine,
- crosslinking the nanofiber layer material by exposure to UV radiation.

Crosslinking can be performed by exposing the nanofiber layer to a temperature of 130°C for about 10 min.

A crosslinking agent tetramethylene glycol dimethacrylate (TEGMA) can be added to the spinning mixture.

The nanofiber layer can be connected, through a lamination process, to another textile composite layer using adhesives or non-adhesive technologies.

The substrate used in the production of nanofibers can be removed during the lamination process or before the composite is applied.

The nanofiber layer can be provided with a protective envelope, which is removed before the wound cover is applied.

The nanofiber layer is standardly infused with bioactive additives such as antimicrobial substances, analgesic substances, antibiotics, chemotherapeutics or enzymes.

The method described above is used to produce a textile composite designed for wound dressing composed of at least one layer, which includes at least one nanofiber layer, while the nanofiber layer is prepared from chitosan chemically modified by methacrylate groups, the structural stability of which is ensured by UV crosslinking after the production of the nanofiber layer.

The nanofiber layer may preferably be made from a solution containing 2 wt% to 10 wt% chitosan and 0.1 wt% to 0.3 wt% PEO, while acetic acid and water are added at a 2:1 ratio to complete the solution.

The nanofiber layer can also be made from a solution containing 4 wt% to 9 wt% chitosan, 0.1wt% to 0.3 wt% PEO and 0.5 wt% to 5 wt% gelatine, while acetic acid and water are added at a 2:1 ratio to complete the solution.

Therefore, the objective of the invention is to prepare a nanofiber layer from chitosan or a mixture of chitosan/gelatine. Before preparing the spinning solution, the used chitosan (raw material) is chemically modified according to methacrylate groups. The modification of chitosan allows the use of UV in order to ensure the stability of the nanofiber structure in an aqueous environment after the production of the nanofiber structure. A nanofiber structure made from modified chitosan and subsequently stabilized by UV radiation is used to make a wound cover.

The method of manufacturing a wound cover consists of connecting a nanofiber

structure with a textile carrier using lamination technology with or without the use of adhesives.

Preparation of nanofiber layers:
The nanofiber layer is prepared from a chitosan, PEO and optionally gelatine solution dissolved in acetic acid and water. The advantage of using this mixture lies in the combination of the positive properties of polymers related to wound healing. Chitosan and gelatine are biodegradable in a number of days. When used to heal wounds, they are partially decomposed.

The nanofiber layer made from chitosan or chitosan/gelatine mixture is unstable in an aqueous environment. In order to stabilize it, the thermal or chemical crosslinking of chitosan is required.

Thermal stabilization takes place at a temperature of about 130°C for about 10 minutes. The thermal crosslinking of chitosan will also stabilize the gelatine for the required time, i.e. for about the 24 hours necessary to start the tissue granulation and re-epithelisation process.

Chemical crosslinking takes place through the chemical modification of the chitosan polymer according to methacrylate groups and the subsequent crosslinking initiated by UV radiation is applied after the production of nanofibers.

The synthesis of the modified chitosan takes place at neutral pH, the modified polymer well soluble in the spinning solution (60% acetic acid) and possible to produce nanofibers from it.

Table 1

Loading of chitosan g/100ml/molar concentration of monomer with -NH_2 group at 80% deacetylation	Volume of GMA up to 100ml dH_2O/resulting concentration	Theoretical molar ratio chitosan monomer/GMA	Experimentally Specified degree of modification of NH_2 groups
5 g (164 mM/monomer)	1.084 ml (82 mM)	2/1	18%

Table 1 contains an example of a chitosan modification process (KitoZyme) by glycidyl methacrylate at neutral pH. The molar concentration of chitosan monomer with an amino group is derived from an 80% representation of chitosan in the product and an 80% chitosan deacetylation level.

The chitosan modification process at a neutral pH is evident from Fig. 1.

The basis of light-activated crosslinking is the formation of chemical reactive groups, the so-called photoinitiator radicals through the absorption of UV radiation, the subsequent cleavage of the double bonds of the methyl methacrylate groups of the chitosan/TEGMA crosslinker with the formation of new radicals. Their repeated reaction with other methyl methacrylate groups results in the covalent crosslinking of chitosan and TEGMA crosslinker (or only chitosan, if TEGMA is not part of the spinning mixture). This cross-linking mechanism is described in detail in the literature dealing with the development of biocompatible hydrogels, such as, for example, hydrogel based on alginate modified by methyl methacrylate. From the point of view of the reaction mechanism, it is similar to the mechanism we anticipate for modified chitosan.

Figure 2 illustrates a possible mechanism of the light-activated cross-linking of chitosan modified by methacrylate: (A) chitosan modified by methacrylate groups; (B) the mechanism of reactive radical formation by UV radiation in the presence of a photoinitiator; (C) the formation of covalent bonds and crosslinking of chitosan; (D) the predicted structure of the resulting nanofiber.
The combination of polymers creates fibres, which have the ability to absorb fluids, resulting in their gelatinization – this creates a humid environment suitable for wound healing and suitable pH in the wound (the pH value of the material is 7.4).

The structure of the nanofiber layer imitates the structure of the extracellular matrix of the tissue, which means that it provides cells with good conditions for granulation (taking place from the edge of the wound towards the wound centre) and subsequent re-epithelisation through skin cells (fibroblasts). The stability of the nanomaterial in a humid environment is 24 to 48 hours. After 72 hours, it is possible to detect the

residues of biodegradable material at the application site.

Brief Description of the Drawings

The invention will be further illustrated by means of drawings, where:

Fig. 1 shows a chitosan modification scheme at neutral pH;

Fig. 2 shows a mechanism of light-activated cross-linking of chitosan modified by methacrylate: (A) chitosan modified by methacrylate groups; (B) the mechanism of reactive radical formation by UV radiation in the presence of a photoinitiator; (C) the formation of covalent bonds and the crosslinking of chitosan; (D) the predicted structure of the resulting nanofiber.

Examples of carrying out the invention

Example 1

Laminate structure: cover layer (substrate) nonwoven fabric spunbond 20-30 g/m^2

A nanofiber layer is prepared from chitosan chemically modified by methacrylate groups, whose structural stability is ensured by the UV crosslinking after the production of the nanofiber layer.

Example 2

Laminate structure: cover layer (substrate) nonwoven fabric spunbond 20-30 g/m^2

A nanofiber layer is made from a solution containing 2 wt% to 10 wt% chitosan and 0.1 wt% to 0.3 wt% PEO, while acetic acid and water are added at a 2:1 ratio to complete the solution.

Example 3

Laminate structure: cover layer (substrate) nonwoven fabric spunbond 20-30 g/m^2

A nanofiber layer is made from a solution containing 4 wt% to 9 wt% chitosan, 0.1 wt% to 0.3 wt% PEO and 0.5 wt% to 5 et% gelatine, while acetic acid and water are added at a 2:1 ratio to complete the solution.

In Examples 2 and 3, the nanofiber layer is bonded to an absorbent layer through an adhesive approved for use in medical devices. This adhesive may be a powder glue (application 3-10 g/m^2) or a fusible mesh - e.g. PO 4605 (10-20 g/m^2) or a hot-melt glue.

Another option for bonding layers is a non-adhesive bonding, e.g. ultrasonic or thermal bonding.

The substrate, which is used to make the nanofiber layer, can be part of the product always removed before application. In other applications, the substrate can be removed during the lamination process and the product is provided with a protective envelope removed prior to application.

The nanofiber layer is applied directly to the wound, the absorbent layer used in Examples 2 and 3 located in the direction out of the wound. The cover is usually fixed at the application spot by means of a secondary cover - adhesive foil or dressing materials. The cover can also be provided with an adhesive system on the edges and on the outside, which ensures its easy application and fixation at the desired location.

The nanofiber layer may contain bioactive additives such as antimicrobial substances, analgetic substances, antibiotics, chemotherapeutics, enzymes, etc.

Claims

1. A method for producing a nanofiber layer based on chitosan in a polyethylene oxide (PEO) solution designed for a textile composite for wound dressing, **characterized in** the following steps:
   - mixing the chitosan monomer with a methacrylate-containing agent in an aqueous medium,
   - subjecting the mixture to filtration, washing it with H_2O and drying it without access to light,
   - crushing the resulting product into powder, a soluble solid phase modified chitosan,
   - subsequently preparing a spinning mixture by dissolving the modified chitosan and polyethylene oxide (PEO) in an aqueous acetic acid solution,
   - applying the spinning solution prepared in this way on a suitably selected substrate,
   - crosslinking the nanofiber layer material.

2. The method according to Claim 1, **characterised in** the following steps:
   - mixing a chitosan monomer with a $-NH_2$ group at 80% deacetylation dissolved in an aqueous environment at a ratio of 5 g chitosan to 10 ml water with glycidyl methacrylate (GMA) at a ratio of 2 mol chitosan to 1 mol GMA, and blending the mixture for 24 hrs.,
   - subjecting the mixture to filtration, washing it by H_2O and drying it for two days without access to light,
   - crushing the resulting product into powder, a solid phase modified chitosan soluble in an acidic aqueous medium,
   - subsequently preparing a spinning mixture by dissolving the modified chitosan and PEO in an aqueous acetic acid solution at the volume ratio of water to acetic acid 2:1 in such a way that the resultant solution contains 2 wt% to 10 wt% chitosan and 0.1wt% to 0.3 wt% PEO,
   - applying the spinning solution prepared in this way on a suitably selected substrate,
   - crosslinking the nanofiber layer material.

3. The method according to Claim 1 or 2, **characterised in that the** crosslinking is performed by exposing the nanofiber layer to UV radiation.

4. The method according to Claim 1 or 2, **characterised in** the following steps:
   - adding gelatine to the solution of modified chitosan and PEO in such a way that the resultant solution contains 4 wt% to 9 wt% chitosan, 0.1 wt% to 0.3 wt% PEO and 0.5 wt% to 5.0 wt% gelatine,
   - crosslinking the nanofiber layer material by exposing to UV radiation.

5. The method according to Claim 4, **characterised in that the** crosslinking is performed by exposing the nanofiber layer to a temperature of 130°C for about 10 min.

6. The method according to Claims 1 to 5, **characterised in that** the crosslinking agent tetramethylene glycol dimethacrylate (TEGMA) is added to the spinning mixture.

7. The method according to Claims 1 to 6, **characterised in that** the nanofiber layer is connected, through a lamination process, to another textile composite layer using adhesives or non-adhesive technologies.

8. The method according to Claim 7, **characterised in that** the substrate used in the production of nanofibers is removed during the lamination process or before the composite is applied.

9. The method according to Claim 8, **characterised in that** the nanofiber layer is provided with a protective envelope, which is removed before the wound cover is applied.

10. The method according to Claims1 to 9, **characterised in that** the nanofiber layer is infused with bioactive additives of the following types: antimicrobial substances, analgesic agents, antibiotics, chemotherapeutics or enzymes.

11. Textile composite for wound dressing consisting of at least one layer containing at least one nanofiber layer, **characterised in that** the nanofiber layer is prepared from chitosan chemically modified according to methacrylate groups, whereas the structural stability is ensured by UV crosslinking after the production of the nanofiber layer.

12. Textile composite for wound dressing according to Claim 11, **characterized in that** the nanofiber layer is produced from a solution containing chitosan from 2 wt% to 10 wt%, PEO from 0.1 wt% to 0.3 wt%, while acetic acid and water at a mixing ratio of 2:1 are added to complete the solution.

13. Textile composite for wound dressing according to Claim 11, **characterized in that** the nanofiber layer is produced from a solution containing 4 wt% to 9 wt% chitosan, 0.1 wt% to 0.3 wt% PEO and 0.5 wt% to 5 wt% gelatine, while acetic acid and water at a mixing ratio of 2:1 are added to complete the solution.

Fig. 1

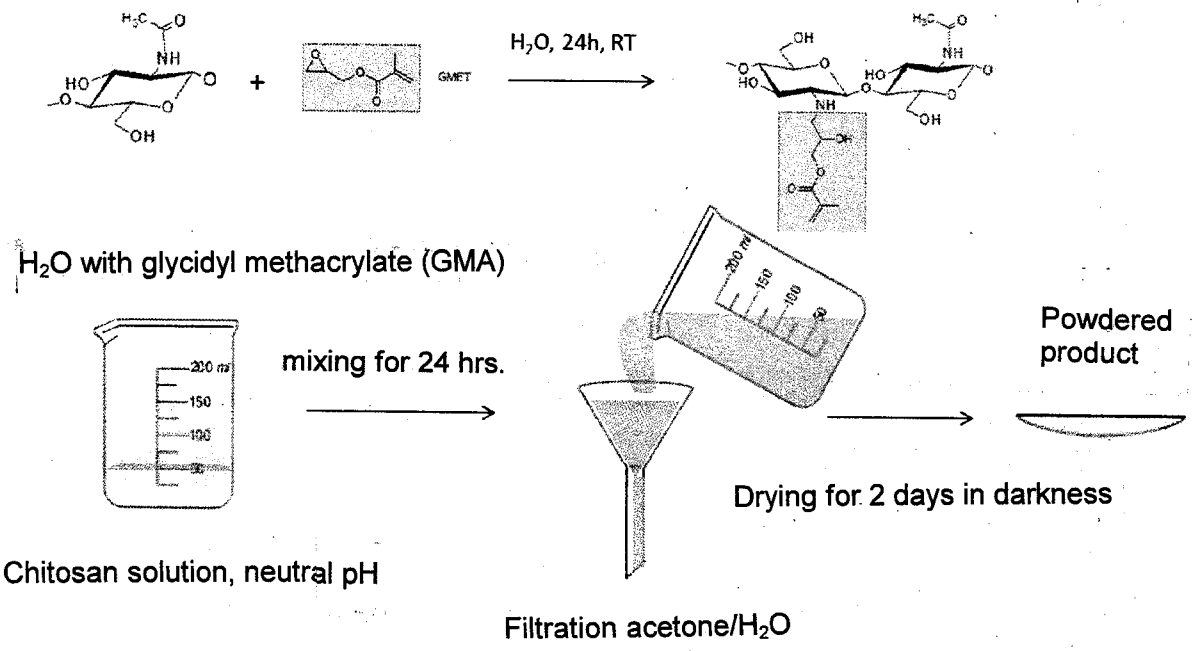
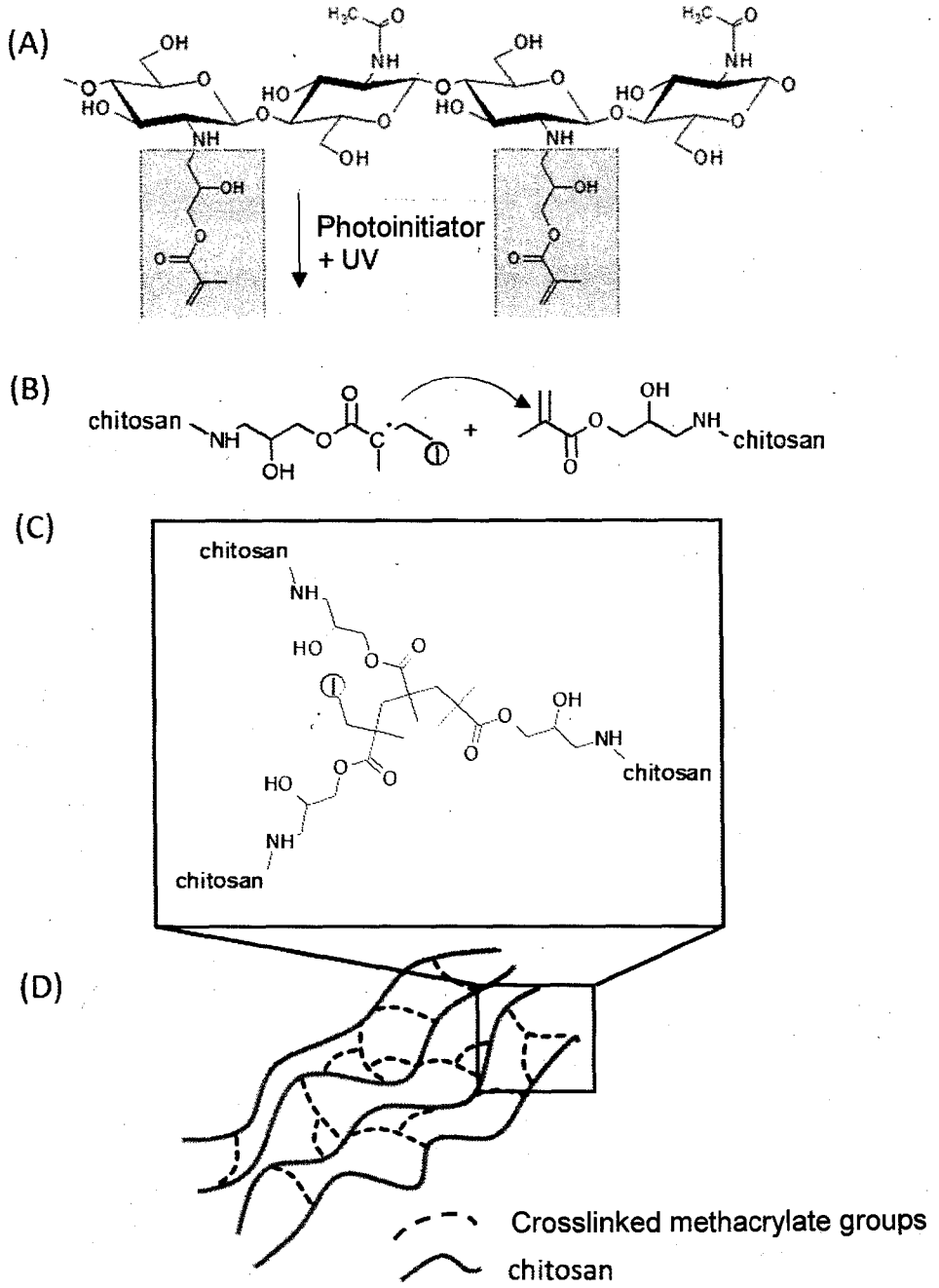

GMET
H_2O, 24h, RT
H_2O with glycidyl methacrylate (GMA)
mixing for 24 hrs.
Chitosan solution, neutral pH
Filtration acetone/H_2O
Drying for 2 days in darkness
Powdered product

Fig. 2


(A)
Photoinitiator
+ UV
(B)
chitosan
chitosan
(C)
chitosan
chitosan
chitosan
(D)
Crosslinked methacrylate groups
chitosan

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2017/000070

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L15/26 A61L15/28 A61L15/44
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)
A61L

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, 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	DATABASE WPI Week 201657 Thomson Scientific, London, GB; AN 2016-28998K XP002778036, & CN 105 536 577 A (UNIV DONGHUA) 4 May 2016 (2016-05-04) abstract -----	1-9, 11-13

☐ Further documents are listed in the continuation of Box C.

☒ 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
19 February 2018	24/04/2018

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	Derrien, Anne-Cécile

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CZ2017/000070

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9, 11-13

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CZ2017/000070

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 105536577 A	04-05-2016	NONE	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-9, 11-13

   method for producing a layer made of nanofiber of chitosan and PEO having methacrylate monomers

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2. claim: 10

   a bioactive layer made of nanofibers of chitosan and PEO
