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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 July 2006 (20.07.2006)

PCT

(10) International Publication Number
WO 2006/076711 A3

(51) International Patent Classification:
*C07K 14/435* (2006.01) *C07K 14/78* (2006.01)
*C07K 14/51* (2006.01) *C12N 15/62* (2006.01)

(21) International Application Number:
PCT/US2006/001536

(22) International Filing Date: 17 January 2006 (17.01.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/644,264 14 January 2005 (14.01.2005) US

(71) Applicants *(for all designated States except US)*: **TRUSTEES OF TUFTS COLLEGE** [US/US]; Ballou Hall, Medford, MA 02155 (US). **UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY OF THE AIR FORCE** [US/US]; AFMCLO/JAZ, 2240 B Street, Room 100, Wright Patterson AFB, OH 45433-7109 (US). **THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS** [US/US]; University of Illinois at Chicago, 1737 West Polk Street, Chicago, IL 60612 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KAPLAN, David, L.** [US/US]; 46 Poond Street, Concord, MA 01742 (US). **HUANG, Jia** [CN/US]; 63 Edward Street, Apt. 2#, Medford, MA 02155 (US). **WONG PO FOO, Cheryl** [MU/US]; 88 B Alder Street, Waltham, MA 02453 (US). **NAIK, Rajesh** [US/US]; 1241 Beech Trail, Dayton, OH 45458 (US). **GEORGE, Anne** [US/US]; 614 Laflin Street, Chicago, IL 60607 (US).

(74) Agents: **RESNICK, David, S.** et al.; Nixon Peabody LLP, 100 Summer Street, Boston, MA 02110-2131 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,

*[Continued on next page]*

(54) Title: FIBROUS PROTEIN FUSIONS AND USE THEREOF IN THE FORMATION OF ADVANCED ORGANIC/INORGANIC COMPOSITE MATERIALS

(57) Abstract: The claimed invention provides a fusion polypeptide comprising a fibrous protein domain and a mineralization domain. The fusion is used to form an organic-inorganic composite. These organic-inorganic composites can be constructed from the nano- to the macro-scale depending on the size of the fibrous protein fusion domain used. In one embodiment, the composites can also be loaded with other compounds (e.g., dyes, drugs, enzymes) depending on the goal for the materials, to further enhance function. This can be achieved during assembly of the material or during the mineralization step in materials formation.

A. Fusion proteins of spider silk and dentin matrix protein 1 (DMP1)

1. Spider silk (CRGD-15mer) - full length DMP1 (Mw = 92.9 kDa)

CRGD-15mer (506 aa) full length DMP1 (474 aa)

2. Spider silk (CRGD-15mer) -C-terminal DMP1 (Mw = 58.9 kDa)

CRGD-15mer (506 aa) C-terminal DMP1 (156 aa)

3. Spider silk (CRGD-15mer) – (CD1 + pA + pB + CD2)

CRGD-15mer (506 aa) (37aa)

B. Fusion protein of spider silk and bone sialoprotein domain (BSP)

1. Spider silk (15mer)- sialoprotein domain (Mw = 42.7 kDa)

15mer (495 aa) BSP (48 aa)

2. Spider silk (CRGD-15mer) – sialoprotein domain (Mw = 48.1 kDa)

CRGD-15mer (499 aa) BSP (48 aa)

WO 2006/076711 A3

LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

**(84) Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*

**(88) Date of publication of the international search report:**
5 July 2007

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/001536

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/435 C07K14/51 C07K14/78 C12N15/62

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)
C07K C12N

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 practical, search terms used)
EPO-Internal, EMBASE, BIOSIS, CHEM ABS Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | MEINEL LORENZ ET AL: "Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds." JOURNAL OF BIOMEDICAL MATERIALS RESEARCH. PART A. 1 OCT 2004, vol. 71, no. 1, 1 October 2004 (2004-10-01), pages 25-34, XP002400266 ISSN: 1549-3296 abstract; figure 2 | 1,2,8 |
| Y | page 33, left-hand column, paragraph 2 - right-hand column, paragraph 1 | 3,9,11, 19 |
| | ----- -/-- | |

[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 document 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 |
|---|---|
| 25 September 2006 | 10 01 2007 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Gurdjian, Didier |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/001536

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | SOFIA S ET AL: "Functionalized silk-based biomaterials for bone formation." JOURNAL OF BIOMEDICAL MATERIALS RESEARCH. JAN 2001, vol. 54, no. 1, January 2001 (2001-01), pages 139-148, XP002400267 ISSN: 0021-9304 | 1,2 |
| Y | abstract<br><br>page 141, left-hand column, paragraph 3<br>page 147; figures 6-9<br>page 140, left-hand column | 3,9,11, 19 |
| X | KONG ET AL: "Silk fibroin regulated mineralization of hydroxyapatite nanocrystals" JOURNAL OF CRYSTAL GROWTH, ELSEVIER, AMSTERDAM, NL, vol. 270, no. 1-2, 15 September 2004 (2004-09-15), pages 197-202, XP005009915 ISSN: 0022-0248 abstract<br>page 198, left-hand column, paragraph 3<br>page 201 - page 202 | 1,2,8 |
| X | HUNTER G K ET AL: "Induction of collagen mineralization by a bone sialoprotein--decorin chimeric protein." JOURNAL OF BIOMEDICAL MATERIALS RESEARCH. 15 JUN 2001, vol. 55, no. 4, 15 June 2001 (2001-06-15), pages 496-502, XP008069209 ISSN: 0021-9304 abstract<br>page 502 | 1,2,8,9, 19 |
| Y | EP 1 413 585 A (E.I. DU PONT DE NEMOURS AND COMPANY) 28 April 2004 (2004-04-28) abstract; claims 1-24; sequences 20-23,60-63 | 3,4,19 |
| Y | WO 02/00016 A (LUMINIS PTY LTD; SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE;) 3 January 2002 (2002-01-03) figures 1,5,7; example 1; sequence 6 | 3,4,19 |
| Y | US 6 291 428 B1 (MACAULAY WILLIAM B ET AL) 18 September 2001 (2001-09-18) column 1; claims 1-4 | 9,11,19 |
| A | EP 0 985 732 A (TERUMO KABUSHIKI KAISHA) 15 March 2000 (2000-03-15) abstract; claims 1-14; figure 1 | 1,2 |

-/--

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2006/001536

## Box 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 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 fee, this Authority did not invite payment of any additional fee.

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.:

1-19

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-19

A fusion polypeptide comprising a fibrous protein domain and a mineralizing domain and correspopnding nucleic acid

1.1. claims: 3-18 fully and 1,2,19 partially

A fusion polypeptide comprising a fibrous protein domain and a mineralizing domain , wherein the fibrous protein domain is obtained from silk , corresponding nucleic acid

1.2. claims: 1,2,19 partially

A fusion polypeptide comprising a fibrous protein domain and a mineralizing domain , wherein the fibrous protein domain is obtained from collagens , corresponding nucleic acid

1.3. claims: 1,2,19 partially

A fusion polypeptide comprising a fibrous protein domain and a mineralizing domain , wherein the fibrous protein domain is obtained from coiled-coiled leucine zipper proteins , corresponding nucleic acid

1.4. claims: 1,2,19 partially

A fusion polypeptide comprising a fibrous protein domain and a mineralizing domain , wherein the fibrous protein domain is obtained from elastins , corresponding nucleic acid

1.5. claims: 1,2,19 partially

A fusion polypeptide comprising a fibrous protein domain and a mineralizing domain , wherein the fibrous protein domain is obtained from keratins , corresponding nucleic acid

1.6. claims: 1,2,19 partially

A fusion polypeptide comprising a fibrous protein domain and a mineralizing domain , wherein the fibrous protein domain is obtained from actins , corresponding nucleic acid

1.7. claims: 1,2,19 partially

A fusion polypeptide comprising a fibrous protein domain and a mineralizing domain , wherein the fibrous protein domain is obtained from tubulins , corresponding nucleic acid

---

2. claims: 20-25

method for forming a fibrous protein inorganic-composite comprisingcontacting a fusion protein comprising a fibrous protein domain and a 'mineralizing' domain , with an inorganic material capable of mineralizing for a sufficient period of time to allow mineralization of the inorganic material, and corresponding product obtained through that method .

---

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/001536

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| T | WONG PO FOO CHERYL ET AL: "Novel nanocomposites from spider silk-silica fusion (chimeric) proteins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. 20 JUN 2006, vol. 103, no. 25, 20 June 2006 (2006-06-20), pages 9428-9433, XP002400268 ISSN: 0027-8424 ----- | 1-19 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2006/001536

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP 1413585 A | 28-04-2004 | NONE | |
| WO 0200016 A | 03-01-2002 | CA 2413449 A1 | 03-01-2002 |
| US 6291428 B1 | 18-09-2001 | NONE | |
| EP 0985732 A | 15-03-2000 | JP 2000125872 A<br>US 6277600 B1 | 09-05-2000<br>21-08-2001 |