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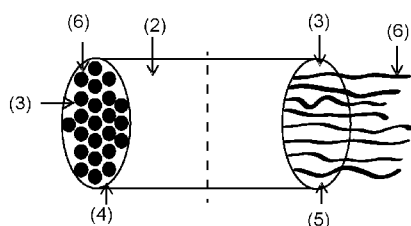
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(54) Title: CONDUIT FOR REGENERATION OF BIOLOGICAL MATERIAL

(1)



(57) Abstract: The present invention relates to a conduit comprising a first material, having 1) a through-going hole, 2) fibers aligned along the longitudinal axis in the through-going hole, each fiber having a diameter in the range 200-2000 nm. The conduit is preferably for regeneration of biological material, even more preferably for nerve regeneration.

Fig. 4



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Conduit for regeneration of biological material

Technical field of the invention

The present invention relates to conduits preferably for regeneration of biological material. The conduits comprise nanofibers arranged inside a hollow material. The invention relates in particular to nerve conduits, processes for producing such conduits and uses thereof.

Background of the invention

Spinal cord injuries (SCI) currently affect more than 240-337,000 people in the United States, with 12,500 new cases each year. The major inhibiting factor to spinal cord injury is not the plasticity of neurons, as within 24 h of injury, neurons seal the damaged axonal membrane, retract the process from the damaged area, and form a new growth cone.

15

In order to address axon guidance in the regenerating spinal cord many groups have studied the application of fibers. Indeed, early attempts at rolling sheets of fibers into a tube for intraspinal implantation have proven to be less optimal due to lack of ingrowth into the densely packed woven fiber sheets, combined with sub-optimal substrate coatings.

20

WO 2006/030 182 discloses a medical device comprising a tubular body having a lumen and a long axis; and a plurality of silk elements laid substantially parallel along the long axis of the lumen of the tubular body.

25

WO 2007/089 259 relates to electrospun fibers deposited on e.g. films. The film may be formed into a tube and the electrospun fiber compositions on a surface of the tube, e.g., the inner surface of the tube. The tubes may be used for nerve regeneration.

30

Hence, an improved conduit for nerve regeneration would be advantageous, and in particular a more efficient and/or reliable method for producing such conduits would be advantageous.

Summary of the invention

By taking advantage of the robust neurite outgrowth associated with aligned nanofibers in vitro, the present invention presents new methods to introduce loosely packed nanofibers into a conduit to promote e.g. glial cell colonization and
5 directed neurite growth. Here we describe the fabrication of aligned, nanofibers and the ability of these nanofibers, to induce rapid nerve outgrowth. Furthermore, by combining such treated nanofibers with hollow tubes (such as polycaprolactone (PCL) tubes), we present a non-woven, aligned, 3D-nanofibrous implant, designed to regenerate e.g. spinal cord injuries in vivo.

10

Thus, an object of the present invention relates to the provision of conduits for regeneration of biological material such as nerves.

In particular, it is an object of the present invention to provide a conduit that
15 solves the above-mentioned problems of the prior art with providing 3D-nerve conduits comprising loosely packed fibers.

Thus, an aspect of the relates to a conduit 1 for regeneration of biological material, said conduit comprising

- 20 - a first material 2, having a through-going hole 3;
 - a plurality of fibers 6
- the fibers 6 being aligned along the long-axis in the through-going hole 3;
 - the fibers 6 having a maximum cross-sectional width in the range
25 200-2000 nm, and
 - the fibers 6 fill 5-90% (by volume) of the through-going hole 3 of the first material 2.

Another aspect of the present invention relates to a process for producing a
30 conduit 1 comprising

- 1) providing a first material 2 having a through-going hole 3;
- 2) providing a plurality of fibers 6, the fibers fiber having maximum cross-sectional width in the range 200-2000 nm;

- 3) threading said fibers through the through-going hole 3;
 - 4) optionally, freeze-drying the first material 2 comprising the fibers 6 distributed in the inner void;
 - 5) optionally cleaving said first material at a desired length,
- 5 thereby providing a conduit comprising,
- a first material 2, having a through-going hole 3;
 - a plurality of fibers 6
 - o the fibers 6 being aligned along the long-axis in the through-going hole 3;
 - 10 o the fibers 6 having a maximum cross-sectional width in the range 200-2000 nm, and
 - o the fibers 6 fill 5-90% (by volume) of the through-going hole 3 of the first material 2.

15 Yet another aspect of the present invention is to provide a conduit 1 according to the invention, for use as a medicament.

Still another aspect of the present invention is to provide a conduit according to the invention for use in regeneration of nerves, muscles fibers, bone, tendons, or
20 cartilage.

In yet an aspect the invention relates a process for producing a conduit 1 comprising

- 1) providing a first material 2 having a through-going hole 3;
 - 25 2) providing a plurality of fibers 6, the fibers fiber having maximum cross-sectional width in the range 200-2000 nm;
 - 3) threading said fibers through the through-going hole 3;
 - 4) optionally, freeze-drying the first material 2 comprising the fibers 6 distributed in the inner void;
 - 30 5) optionally cleaving said first material at a desired length,
- thereby providing a conduit comprising,
- a first material 2, having a through-going hole 3;

- a plurality of fibers 6
 - o the fibers 6 being aligned along the long-axis in the through-going hole 3;
 - o the fibers 6 having a maximum cross-sectional width in the range 200-2000 nm, and
 - o the fibers 6 fill 5-90% (by volume) of the through-going hole 3 of the first material 2.

Brief description of the figures

10 Figure 1

SEM of electrospun silk fibroin nanofibers (A). Image analysis for mean fiber diameter was determined to be $0.557 \pm 0.181 \mu\text{m}$ and $0.563 \pm 0.180 \mu\text{m}$ measured before and after dissolving PEO from fibers. FFT alignment analysis of aligned nanofibers (B).

15 Figure 2

Immunosorbent analysis of HSPG and CSPG deposition on PDL and laminin coated nanofibers.

Figure 3

Heparan sulfate increases neurite outgrowth on coated silk fibroin nanofibers.

- 20 (A) Representative images of cells seeded on PDL and laminin coated substrates with or without HSPG coatings assessed for neurite outgrowth (neurites longer than cell body) and length by CLSM imaging and analysis (scale bar represents $100 \mu\text{m}$).

- (B) The length of the longest neurite per cell was measured after 36 h and
 25 significantly longer in HSPG samples ($n > 192$) compared to controls ($n = 56$). X-axis = $\mu\text{g/ml}$; Y-axis = Average neurite length.

- (C) The number of cells extending neurites was also significantly increased on HSPG coated substrates. $*P < 0.05$, $****P < 0.0001$. There were no significant differences ($P > 0.05$) between the number or length of neurites cultured with
 30 higher concentrations of HSPG. X-axis = $\mu\text{g/ml}$; Y-axis = Percent of neurons with axons.

Figure 4

Figure 4 is a schematic overview of a conduit according to the present invention. (1) conduit according to the invention, (2) first material, (3) through-going hole, (4, 5) first and second opening in the through-going hole, and (6) plurality of
5 fibers.

Figure 5

Fabrication of aligned silk nanofiber conduits. (A) Wet banded aligned fibers before threading inside of a PCL tube. (B) Macro image showing the length of the nerve conduits. (C) SEM of alignment and packing of uncoated nanofiber conduits
10 after freeze-drying. (D) High magnification of inset. (E) Conduit and nanofibers coated with PDL and laminin, and with additional HSPG (F) after freeze-drying.

Figures 6 and 7

SEM Images of non freeze-dried fibers (6) in tubes (2).

15 Figures 8 and 9

SEM of alignment and packing of (6) nanofibers coated with PDL and laminin, and with additional HSPG (F) after freeze-drying. Embodiment with 80% packing ratio and hierarchical arrangement of nanofibers in aligned fascicles with thicknesses ranged from 5 – 20 μm .

20

The present invention will now be described in more detail in the following.

Detailed description of the inventionConduit for regeneration of biological material

25 The inventors have produced an improved conduit (preferably a nerve conduit). Thus, an aspect of the relates to a conduit 1 for regeneration of biological material, said conduit comprising

- a first material 2, having a through-going hole 3;
- a plurality of fibers 6
 - 30 ○ the fibers 6 being aligned along the long-axis in the through-going hole 3;
 - the fibers 6 having a maximum cross-sectional width in the range 200-2000 nm, and

- the fibers 6 fill 5-90% (by volume) of the through-going hole 3 of the first material 2.

Volume

In the present context, the volume relates to the volume-percentage (vol-%) the fibers constitute of the hole 3 (void) in the first material. Thus, the volume may be determined by e.g. electron-microscopy of a cross-section of a conduit according to the invention. The volume the fibers are taking up in the void can then be determined. It is to be understood that the volume may be determined when the conduit is in a dry or wet state. Other means for determining the volume may be by the use of confocal microscopy or histological methods such as by embedding in resin and imaging a section using microscopy.

The through-going hole 3 may be described in different ways. Thus, it may also be defined as an inner void and two (opposite) openings 4, 5. Similar the wording "aligned along the long-axis" in the through-going hole, may be described in different ways. Thus, in another embodiment the fibers are aligned parallel to each other or substantially parallel to each other, and perpendicular or substantially perpendicular to the two openings 4, 5 of the first material.

The technical effect of the fibers is that e.g. nerve cells may migrate from one end of the hole towards the other end by using the fibers as directional guides. Thus, parallel may also be defined as the fibers running from one opening in the first material to another opening. Thus, the fibers are also through-going. In example 3, fast nerve growth on thin fibers according to the present invention is described and in example 4 conduits according to the present invention are further described. In figure 4 a schematic overview of a conduit according to the invention is presented.

It may be advantageous to increase the surface area of the fibers in the hole to improve nerve ingrowth along the fibers. Thus, in yet an embodiment, said fibers 6 are distributed evenly or substantially evenly throughout the through-going hole 3 of the first material 2. In a further embodiment, said fibers fill 20-80% (by volume) of the inner void, such as 30-80%, such as 40-80%, such as 60-80% preferably, such as 40-70%, or more preferably such as 40-60% of the inner void. The remaining space in the hole (not constituted of fibers) leave space for growing nerve cells (when the conduit is for regeneration of nerves or nerve cells).

The first material of the conduit according to the present invention may comprises different materials. Thus, in an embodiment, the first material 2 comprises Polycaprolactone (PCL), Polyglycolic Acid (PGA), PLA (PLLA), PLGA

5 (Polylactideglycolic acid), peptide amphiphile solutions, PEG (Polyethyleneglycol), PLA-b-PEG-b-PLA, collagen, elastin, chitosan, ϵ -caprolactone and ethyl ethylene phosphate (PCLEEP), poly(ethylene-co-vinyl acetate) (PEVA), crosslinked gelatin, fibrin, polydioxanone (PDO), PCL+PLA as copolymers or blends, silk, or combinations thereof. In example 4, the first material is made of PCL.

10

In yet an embodiment, the first material 2 comprises one or more polymer types and co-polymers comprising polyesters including Polycaprolactone, Polyglycolic Acid (PGA), Polylactic Acid (PLA), Polycaprolactone-co-lactic acid, Poly(D, L-lactide-co- ϵ -caprolactone) [PDLLA/CL], Polyhydroxyalkanoates (e.g. Poly(3-hydroxybutyrate), Poly(4-hydroxybutyrate), Poly(3-hydroxyvalerate), Poly(3-hydroxyhexanoate), Poly(3-hydroxyoctanoate), Poly(3-hydroxydecanoate)), cross-linked Polyester elastomers: poly(polyol sebacate) (where the alcohol can comprise Glycol; Glycerol; Erythritol ; Threitol; Arabitol ; Xylitol; Mannitol).

15

Polymers can be blended so they form advantageous eutectic or non-eutectic

20 systems in advantageous embodiments of the first material and/or in the fibers.

In yet an embodiment, the first material 2 comprises:

Synthetic DNA, and/or

Naturally derived polymers

25

- from animals such as chitosan, cross-linked hyaluronic acid, Hagfish slime, mussel byssus protein, thermally crosslinked gelatin, chemically cross-linked gelatin, complex coacervated hyaluronic acid-gelatin, the family of collagens and derivatives, recombinant or animal derived keratin types 1 to 20 including derivations and combinations thereof, recombinant or animal

30

- fibrillin types 1 to 4 including derivations and combinations thereof; or
- from plants such as lutein, Zein, cross-linked hydroxyethylcellulose, crosslinked hydroxypropylcellulose, crosslinked methylcellulose, gellan, xanthan gum and/or derivatives.

In further advantageous embodiments, the first material and/or the fibers comprise composites with embedded particulates. These particulates include calcium phosphate nano- and microparticles, bioglass nano- and microparticles, oriented or randomly arranged carbon nanotubes, buckyballs, silicates (clays).

5

In other advantageous embodiments, the first material comprises decellularized tissue of animal or human origin. Examples include decellularized tubular structures such as veins and venules, lymph vessels, arteries and arterioles.

10 Similar, the fibers of the conduit according to the present invention may comprise different materials including those listed for the first material. Thus, in an embodiment the fibers comprises silk, silk fibroin, Polyglycolic Acid (PGA), PLLA, PCL, Peptide amphiphile solutions, or mixtures thereof. In example 4, the fibers are electrospun fibers of fibroin.

15

The fibers will preferably have a round cross-section, but other shapes may also be used. Thus, in embodiments fibers are in the form of fiber ribbons, round or oval, preferably round. In further advantageous embodiments, the fibers are hollow.

20

In yet an embodiment, the fibers are arranged in secondary, tertiary, and quaternary structure in the first material. These structures may comprise bundles with cross-sectional aspect ratios of 1:1 to 100:1; 100:1 to 1000:1, twists, braids, and/or weaves. In yet an embodiment the second material is arranged in

25 combinations of said secondary structures.

The fibers may be constituted of different fiber types. Thus, in a further embodiment said mixtures thereof are fibers with one fiber encapsulating another fiber.

30

The first material and the fibers may be combined in preferred combinations. Thus, in an embodiment said first material comprises PCL and the fibers comprises silk fibers, OR the first material comprises collagen and the second material comprises silk, OR, the first material comprises PCL and the fibers

35 comprises PGA, OR the first material comprises PGA and the fibers comprises

PGA, OR the first material comprises PCL and the fibers comprises PCL fibers, OR the first material comprises PGA and the fibers comprises silk fibers, OR the first material comprises silk and the fibers comprises silk fibers. In example 4 the first material is PCL and the fibers are silk fibroin.

5

Silk derived from Bombyx Mori silk worms is composed of two components; sericin, the outer coating of the fibers, is responsible for the material's sticky properties; and silk fibroin, (SF) a repeating beta sheet of $-(\text{Gly-Ser-Gly-Ala-Gly-Ala})-$ which forms the peptide core. Complete removal of the sericin coating
10 (degumming) is essential to avoid immunogenic complications upon implantation of this biologically amenable material. Consequently, purified silk fibroin has been approved as an implantable biomaterial by the FDA.

Different types of silk may be used to form the fibers of the conduit. Thus, in an
15 embodiment, the silk is synthetic or recombinant silk or natural silk, such as silk from mulberry silkworm silk, non-mulberry silkworm silk, spider dragline silk, and/or bee silk. In a more specific embodiment, the fibers are made of silk fibroin. Though silk fibers are used as an example of fibers in example 4, other non-biological fiber types may be preferred. Thus, in an embodiment the fibers are not
20 silk fibers.

Since the conduit according to the invention has completed its purpose when e.g. a nerve has re-generated it would be preferable if a clinician would not have to remove the conduit afterwards. Thus, in a preferred embodiment the conduit is
25 biodegradable and/or bio-absorbable. In that respect, the conduit according to the invention may be considered a medicament and/or composition by not be re-usable.

The inner width of the hole may be adjusted to the exact purpose. For example,
30 the width or diameter will depend on e.g. the size of the nerve(s), which it is intended to support during regeneration. Thus, in an embodiment the inner width of the hole (parallel to the orifice of the openings /cross-sectional width) is in the range 0.5 mm to 1 cm, such as in the range 0.5 mm to 6 mm, such as in the range 0.5 mm to 4 mm, such as in the range 0.5 mm to 2 mm, such as in the

range 1 mm to 8 mm, such as in the range 2 mm to 8 mm, or such as in the range 3 mm to 6 mm.

Diameter

- 5 In the present context, the terms "diameter" or "width" relate to the diameter or width of a cross-section of the part in question. For non-round sections, the diameter or width relates to a maximum diameter or width of the cross-section. For example, if the fibers are ribbon-shaped, the diameter relates to the maximum diameter/width of the ribbon (cross-section).

10

Similar, the outer width of the conduit may be adjusted to the exact purpose. For example, the width or diameter will depend on e.g. the size of the nerve(s), which it is intended to support during regeneration, the space available, and/or requirement for strength of the conduit. Thus, in an embodiment the outer width

- 15 of the conduit is in the range 0.8 mm to 20 mm, such as in the range 1 mm to 20 mm, such as in the range 5 mm to 20 mm, such as in the range 10 mm to 20 mm.

The length of the conduit may also be adapted to the space or injury it has to

- 20 cover. Thus, in an embodiment, the conduit has a length in the range 5 mm to 10 cm, such as 1-10 cm, such as 3-10 cm, such as 5-10 cm, such as 1-7 cm, such as 1-5 cm, or such as 1-3 cm. It is to be understood that the length relates to the distance between the orifices of the openings in the through-going hole 3.

- 25 The maximum diameter (or width) of the fibers may vary. Thus, in an embodiment maximum diameter of the fibers is in the range 200-800 nm, such as 300-800 nm, such as 400-800 nm, such as 500-800 nm, such as 200-600 nm or such as 400-600 nm, or such as 700-900 nm. It is believed that nerve growth is improved when the maximum diameter (or width) is in these ranges compared to
- 30 the much larger fibers used in e.g. WO 2006/030 182. In example 1, diameters of fibers around 500 nm are produced.

It will of course be advantageous to have the conduit in a form, which makes

nerve grafting easy. Thus, in an embodiment at least some of the fibers protrude

- 35 from at least one end of the first material, such as both ends, with a distance of

0.1 mm to 1 cm, such as 2 mm to 1 cm, such as 5 mm 1 cm. By having the fibers protruding from the conduit nerve grafting by a clinician is easier/faster.

The exact number of fibers positioned in the hole may vary tremendously
5 depending on the size of the hole and the exact size of the fibers. In an embodiment, the number of fibers in the hole is in the range $10^3 - 10^8$, $10^5 - 10^8$, or such as $10^6 - 10^8$.

The exact shape of the conduit may of course also vary. In a further embodiment,
10 said conduit is tube-shaped, oval, round, polygonal or square. Preferably, the conduit is tube-shaped.

As described previously, previously produced nerve conduits have been produced by rolling a sheet of film into a tube. Such forming will result in non-smooth
15 surfaces both on the inside and outside of the tube. In an embodiment of the present invention, the inner and/or outer side of the first material is smooth or substantially smooth. This is a result of the described production process described for the present invention.

20 To improve e.g. nerve growth and acceptance by surrounding tissue, it may be advantageous to coat the first material and/or the fibers. Thus, in an embodiment the conduit comprises one or more biologically active substances. In a further embodiment, the biologically active substances are selected from the group consisting of growth factors, cytokines, antibiotics, immunosuppressants, steroids,
25 hormones, non-steroidal anti-inflammatory drugs (NSAIDs), peptide sequences such as RGD, nucleotides, small interfering RNAs, antisense RNAs acrylic acid, and/or heparin sulfate proteoglycans. In yet a further embodiment the fibers are coated with heparin sulfate proteoglycan (HSPG), laminin, and/or NGF (Nerve growth factor). In example 2, experiments with HSPG are conducted. IN yet
30 another embodiment, a fraction of the fibers are coated with heparin sulfate proteoglycan (HSPG), laminin, and/or NGF (Nerve growth factor). However, without being bound by theory, these explicit coatings are not considered essential for in vivo uses, where systemic factors may also stimulate growth. For in vitro uses this may still be very relevant. It has been found that antisense

RNA's are more easy to introduce than interfering RNAs, thus antisense RNA's are the preferred RNA type.

In yet a further embodiment, the biologically active substances are coated on the
5 fibers, and/or on the outer surface of the first material and/or on the inner surface of the first material.

The conduit may also further comprise cells. Thus, in an embodiment the conduit further comprises living cells, preferably in the inner void of the first material. In
10 yet an embodiment the cells are nerve cells, such as Schwann cells and/or olfactory ensheathing glia (OEG), and/or adipose stems cells, and/or mesenchymal stem cells and/or induced pluripotent stem cells.

Because of the described production process, some of the fibers will be aligned in
15 opposite directions. Thus, in a further embodiment, a part of the fibers 6 are aligned in opposite directions, such as half of the fibers.

In another embodiment a portion of the fibers 6 are aligned at angle.

20 Another consequence of the production process is that at least some of the fibers are not in contact with the first material but will be distributed through-out the hole 3. Thus, in an embodiment, at least some of the fibers 6 are not in direct contact with the first material 2. This is not the case for conduits disclosed in e.g. WO 2007/089 259, which is formed by rolling a mat or film. Thus, in yet an
25 embodiment the first material 2 is not a rolled film or mat.

In the example section conduits particularly suitable for nerve-regeneration is presented. However, other uses may also be foreseen. Thus, in an embodiment, the conduit is for regeneration of biological material selected from the group
30 consisting of muscles fibers, bone, tendons, and/or cartilage, preferably nerves. It is also to be understood that the use is preferably for regeneration of mammalian biological material, such as humans.

As described above, the conduit according to the present invention comprises
35 space in the hole for invading/growing nerve cells. Thus, in yet an embodiment,

the remaining void in the first material is filled with a gel, and/or liquid such as a saline or nutrient solution. Such fluid or gel may prevent packing of the fibers. In addition, the fluid may also serve to suspend the fibers after dehydration or freeze-drying. Remaining void refers to the space not occupied by the fibers. This
5 space leaves room for nerve ingrowth.

The conduit according to the invention may have one or more longitudinal seams either thermally fused, solvent bonded, stitched with a biomaterial, glued or cross-linked together, glued with an advantageous material different from the
10 tube. In another advantageous embodiment, fibers are placed on a sheet of first material, which is then wrapped around the fiber and fused with above means.

It may also be advantageous if nutrients could easy access the hole of the conduit e.g. after insertion in the body and or during in vitro use. Thus, in an
15 embodiment, the first material is porous. The porosity allows for diffusion throughout the conduit. Diffusion throughout the conduit is to be understood in the meaning, but not limited to, that substantially the entire conduit is diffused, such as for example about 99%, such as about 90%, such as about 80%, such as about 70% of the conduit is diffused. Transport mechanisms (diffusion and
20 convection) can be guided through openings in the conduits (large openings + smaller openings due to the porosity. In the present context porous relates to pores having a maximum width in the range 0.01-10 μm , such as in the range 0.1-10 μm , such as within a range 0.1-5 μm such as in the range 1-5 μm (leaving room for influx of nutrients etc. but avoids wrong cell migration).
25 As is evident from the disclosure of the present invention, the conduit may be used as a medical implant. Thus, in an embodiment the conduit is suitable for implantation in a mammal, such as a human. In another embodiment, the conduit is sterile.

30 **Medical uses**

The conduit according to the present invention may also be considered a medicament of medical composition by being expended in the process of use and have only a once for all utility. This is underlined by the preferred biodegradability described above. Thus, an aspect of the invention relates to a conduit 1 according
35 to the invention, for use as a medicament.

In yet an aspect the invention relates to a conduit according to the invention for use in regeneration of nerves, muscles fibers, bone, tendons, or cartilage. In a preferred embodiment, said use is for nerve regeneration, such as regeneration of
5 peripheral nerves and/or spinal nerves.

Ex vivo uses

The conduit according to the present invention may also find purposes ex vivo. Thus, an aspect of the invention relates to the use of the conduit 1 according to
10 the present invention for ex vivo growth of nerves, muscles fibers, bone, tendons, or cartilage. In a preferred embodiment, the use is for nerve regeneration, such as peripheral nerves and/or spinal nerves.

Process for producing conduit

15 Conduits according to the present invention may be produced in different ways. An aspect of the present invention relates to a process for producing a conduit 1 comprising

- 1) providing a first material 2 having a through-going hole 3;
- 2) providing a plurality of fibers 6, the fibers fiber having maximum cross-
20 sectional width in the range 200-2000 nm;
- 3) threading said fibers through the through-going hole 3;
- 4) optionally, freeze-drying the first material 2 comprising the fibers 6 distributed in the inner void;
- 5) optionally cleaving said first material at a desired length,
- 25 thereby providing a conduit comprising,
 - a first material 2, having a through-going hole 3;
 - a plurality of fibers 6
 - 30 ○ the fibers 6 being aligned along the long-axis in the through-going hole 3;
 - the fibers 6 having a maximum cross-sectional width in the range 200-2000 nm, and
 - the fibers 6 fill 5-90% (by volume) of the through-going hole 3 of the first material 2.

The inventors have identified a particular process allowing for the preparation of thin fibers. Thus, in an embodiment the fibers 6 provided in step 2) are produced by electrospinning. In yet an embodiment the fibers are produced by co-spinning
5 two or more solutions. In yet a further embodiment electrospinning is "melt spinning".

In yet a further embodiment the fibers are produced by centrifugal spinning.

- 10 A first obstacle when trying to produce conduits according to the present invention is the provision of the thin fibers in a form free from the surface on which they have been deposited, which allows for subsequent 3D-positioning of fibers 6 in the hole in the first material. Thus, in yet an embodiment, the electrospun fibers (or centrifugal spun fibers) are collected on a rotating drum. In yet a further
15 embodiment, the process comprises collecting (e.g. around two rods) the electrospun (or centrifugal spun) fibers, thereby providing fibers free from the rotating drum. In yet an embodiment the electrospun fibers are collected around two rods.
- 20 In an alternative embodiment, the electrospun fibers are deposited directly on a charged collection device comprising two rods, thereby providing fibers aligned parallel between the rods. In yet a further embodiment, the charged collection device is positively or negatively charged.
- 25 A further obstacle when trying to produce conduits according to the present invention is the positioning of the collected fibers in the hole in the first material. Thus, in an embodiment, after having released the electrospun (or centrifugal spun) fibers from a surface (such as a rotating drum), the process further comprises wrapping a thread around the fibers and threading said fibers 6 through
30 the void of the first material according to step 3 in the process. In a specific embodiment, said thread wrapped around the fibers 6 is made of a polymer such as PCL.

The electro-spinning may be further improved (for some materials such as silk),
35 by changing the viscosity of the fiber material. Thus, in an embodiment, during

electrospinning, the fiber is mixed with a viscosity-increasing agent such as polyethylene oxide (PEO).

The fibers made during electrospinning (or centrifugal spinning) may have
5 different widths depending on the particular use. Thus, in an embodiment the diameter of the fibers are adjusted by increasing or decreasing the distance between the emitter and collector devices during spinning.

In an alternative aspect of the invention, the conduit is produced by wrapping the
10 first material around the plurality of fibers and sealing the first material.

Product obtained/obtainable by process

In an aspect, the invention relates to a scaffold obtainable/obtained by process according to the invention.

15

It should be noted that embodiments and features described in the context of one of the aspects of the present invention also apply to the other aspects of the invention.

20 All patent and non-patent references cited in the present application, are hereby incorporated by reference in their entirety.

The invention will now be described in further details in the following non-limiting examples.

25 Examples

MATERIALS AND METHODS:

Synthesis and Preparation of Silk Fibroin Nanofibers

Silk fibroin was prepared as described previously (Rockwood, D. N. et al. Materials
30 fabrication from Bombyx mori silk fibroin. Nat. Protocols 6, 1612-1631 (2011)). Briefly, Bombyx Mori silkworm cocoons were cut open, cleaned and boiled in 0.02M Na₂CO₃ for 30 minutes. The fibers were rinsed and dried overnight. Dry silk was packed in a small beaker and 9.3M LiBr was layered on top and heated to 60°C for 4 hours. The resulting solution was added to a 3500 MWCO dialysis

cassette and dialyzed against ultrapure water for 48hrs. The silk solution was cleared by centrifugation. To determine solution's concentration an exact volume is allowed to dry completely so that the resulting weight can be used to calculate the % w/v. If necessary, silk fibroin was concentrated against poly ethylene oxide (PEO, 900kDa; Sigma-Aldrich, St. Louis, Missouri, USA).

Electrospinning of silk fibroin nanofibers was performed using a solution of 7.2% silk, and 1% PEO in ddH₂O loaded into a syringe fitted with a metallic needle (16 gauge) and extruded at a rate of 0.9ml – 2 ml/hr. The syringe was fixed horizontally on the syringe pump (model AL-1000-220Z, World Precision Instruments), and an electrode of high-voltage power supply (Gamma High Voltage Research) was clamped to the metal needle tip. The flow rate was 0.9 mL/h, and the applied voltage was 7.3-7.7 kV. The tip-to-collector distance was set to 13 cm, and a grounded collector (r = 4,22 cm w = 14,5) rotating at 4300 rpm was covered by a piece of clean aluminum foil with double sided tape to adhere 13 mm coverslips for fiber collection. Temperature and humidity ranged between 20-22°C and 30-60%, respectively. The collection time was 40 min in order to obtain a single layer of fibers with a density around 40 fibers per 100 µm.

Silk fibroin beta-sheet structure was induced by treatment with 90% (vol/vol) methanol/water for 20 min, followed by washing with ultrapure water on a reciprocating shaker overnight to remove PEO. Nanofibers were treated with 100 µg ml⁻¹ Poly-D-Lysine (Sigma-Aldrich) for 30 minutes at room temperature followed by washing in ddH₂O. Samples were subsequently treated with 1 µg ml⁻¹ laminin (Sigma-Aldrich) for 1 hr at room temperature, washed, and treated with varying doses of heparan sulfate proteoglycan (H4777, Sigma-Aldrich) for 1 hour at room temperature followed by washing with phosphate buffered saline (PBS).

Alternatively, the fibers can be collected using two grounded metal rods with spacing between the two rods twice the length of the resulting tube conduit.

Characterization of Nanofibers

The fiber morphology was examined by environmental scanning electron microscopy (FEI, Nova 600 NanoSEM) at 5 kV and chamber pressure 60 Pa. Environmental SEM allowed the fibers to be imaged without further preparation.

Fiber diameter is based on measurements of 150 fibers both before and after treatment, using ImageJ.

SEM samples of tubes containing nanofibers were freeze-cleaved using liquid
5 nitrogen to provide a cleanly cut surface of the silk fibres and PCL conduit.

Rat Dorsal Root Ganglia Preparation

DRG's were extracted from Wistar neonates p4-5 (any gender) and isolated using enzymatic and mechanical procedures with modifications 45. Briefly, animals were
10 decapitated and spines were excised and split down the midline to expose the interior of the spinal canal. Ganglia were removed and nerve roots trimmed, before two rounds of 0.125% w/v collagenase type IV treatment (Worthington Biochemical Corp., Lakewood, NJ, USA) for 1 hour at 37°C. Followed by 30 minutes of 0.25% Trypsin (Worthington Biochemical Corp.) digestion at 37°C.
15 Trypsin is neutralized with 33% fetal bovine serum, washed and triturized in a flame polished Pasteur pipet. DRG are cleared of debris by layering cells on 15% bovine serum albumin (BSA) in L15 media and centrifuging at 300 x g for 10 minutes. DRG are cultured in Neurobasal (Thermo-Fisher) supplemented with B-27® (Thermo-Fisher), penicillin/streptomycin (Thermo-Fisher) and 1 ng/ml NGF
20 (Abd Serotec, Oxford, UK) and seeded at 2 X 10⁴ cells/13mm coverslip.

Confocal Scanning Laser Microscopy

Coverslips were washed in PBS and treated for 10 minutes in 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) and washed.
25 Samples were permeabilized and blocked in PBS containing 0.25% Triton-X100 (Sigma-Aldrich) and 5% donkey serum (Sigma-Aldrich) for 45 minutes. Samples were incubated overnight at 4°C with either, 1:500 mouse monoclonal IgM antibodies to neurofilament heavy chain (OMA1-06116, Thermo Fisher, Waltham, MA, USA), 1:200 mouse monoclonal IgM antibodies to chondroitin sulfate
30 (ab11570, Abcam, Cambridge, UK), or 1:200 rat monoclonal IgG2a antibodies to heparan sulfate (ab2501, Abcam) diluted in 5% goat serum. After washing, secondary goat anti-mouse IgM conjugated to DyLight 594 (ab98676, Abcam) or Goat anti-rat IgG conjugated to DyLight 650 (ab98389, Abcam) was diluted 1:1000 and incubated for 45 minutes at room temperature. The coverslips were
35 washed, de-salted and dried before mounting with prolong gold® (Thermo-

Fisher). A LSM 700 confocal laser scanning microscope (Zeiss, Germany) using a 63x/1.4 oil objective with 8-bit depth. Fluorescence signals were captured within the dynamic range of the signal intensity. Silk fibers were imaged by increasing the 405 nm laser power to enhance auto-fluorescence at 410/20 nm. Tile scans
5 with 12% overlap were stitched together using Zen Blue software (Zeiss). Contrast and brightness was adjusted by ImageJ.

Dorsal Root Ganglia Neurite Analysis

To analyze the effects of HSPG coated silk nanofibers on DRG neurite outgrowth,
10 cells were cultured for 36 h after seeding. Neurocan (5800-NC-50, R&D systems, Minneapolis, MN, USA) was added to culture media 12hr after seeding to prevent DRG attachment inhibition. The number and length of neurites was assessed by counting cells with neurites longer than their cell body as positive for outgrowth, and by measuring the longest neurite extended from each cell with the simple
15 neurite tracer plugin for Image J 44.

Preparation of Polycaprolactone Tubes

Solid wall polycaprolactone tubes were made by filling cylindrical open-ended molds with polycaprolactone melt at 115 ° C. The length and internal diameter of
20 the molds was 20 mm and 2.30 mm, respectively. Purging with compressed air through one end of the mold, left behind a tube on the inside of the tube wall. Following a cool-down to 2°C, these coarse tubes were released from the molds, clamped in either ends with pens, and were manually stretched up 110 mm or until the necking zone spanned the entire length of the tube. SEM of the PCL
25 tubes showed that the method was highly reproducible and yielded consistent internal lumen diameters and wall thickness of 1.6 mm and 200 µm, respectively.

Preparation of Nanofibrous Conduits

By density analysis, a defined amount of nanofibers from the collection surface is
30 gathered and threaded through a capillary tube. The appropriate density is gathered by wrapping the fibres in a band around the tips of a 50 mm wide winder made from 1.0 mm stainless steel rod. Stationary fibres suspended between winder prongs are now able to be treated in various solutions with the final washes containing 100 mM sucrose to stabilise the proteins for freeze drying
35 and storage. The suspended fibre bundle on the winder were threaded through

the PCL tubes by looping a double butted PCL fiber around the middle of bundle and then inserting the thick ends into the tube. The thicker section of PCL fibre is stiff enough to be pushed through the whole length of the tube without piercing or destroying the lumen like a needle (Fig 5a). The thinner midsection of the fiber
5 allows for pulling relatively thick bundles of silk nanofibers through. Once the PCL fibre holding the nanofibers has been pulled to the opposite end of the tube, it is severed. The resulting tubes containing the fibres were placed in a tube, snap frozen in a dry ice and ethanol bath, and freeze dried. Before use in animals, the tube is rehydrated and cleaved at lengths suitable for surgical manipulations.

10

Data Analysis and Statistics

All experiments were performed in at least biological triplicate. Unpaired student's t-tests were used infer result significance for neurite length in HSPG, and Chi-squared tests were employed for neurite outgrowth.

15

Example 1 - Characterization of Nanofibers

The aligned electrospun silk fibers had a mean diameter of $0.557 \pm 0.181 \mu\text{m}$ and $0.563 \pm 0.180 \mu\text{m}$ measured before and after dissolving PEO from fibers.

20 Example 2 - Nanofiber Coating Validation

The confirmed presence of the proteoglycan coatings, and ability for the Neurocan treatments to deposit and remain on the surface of the scaffolds was evaluated by immunochemistry and CLSM (Fig. 2). Laser power and gain was set using negative primary antibody controls to ensure no background interference from
25 unspecific secondary antibody binding was present in primary antibody hybridized samples. CLSM images showed clear fluorescent signals associated with HSPG ($25 \mu\text{g ml}^{-1}$) and/or CSPG ($25 \mu\text{g ml}^{-1}$) treatment.

Conclusion

30 This study shows that the proteoglycans are able to stably deposit on the PDL ($100 \mu\text{g ml}^{-1}$) and laminin ($1 \mu\text{g ml}^{-1}$) coated nanofiber surfaces.

Example 3 - Neurite Outgrowth Studies

The additional coating of HSPG increased the number of DRG's exhibiting neurite outgrowth by 33% (Fig. 3c) for a total of 73-77% of neurons positive for neurites ($P < 0.05$). The number of neurite positive DRG's in the control sample ($n = 56$) is lower than those analyzed within the same area scanned for HSPG treated samples ($n > 192$), but had no effect on the results' significance due to the robust response to the treatment. Neurite lengths were, on average, 100 μm longer on substrates coated with HSPG when compared to controls (Fig. 3b). The longest measured individual neurites measured well over 1 mm within just 36 h. Such neurons had optimal conditions for rapid extension. Indeed, once in contact with the nanofiber, the alignment and strong physical guidance due to the optimal nanofiber diameters lowers the probability of branching and thereby promotes the investment of energy into fewer growth cones.

In order to assess if the HSPG coating was capable of desensitizing neurons to the neurite repulsive effects of CSPG's, neuron cultures were treated with increasing concentrations of neurocan. As substrate bound CSPG's have been shown to reduce DRG attachment in vitro, it was important to ensure cell attachment to the substrates, therefore neurocan was only added to cultures 12 h after seeding, and outgrowth was assessed 36 h post treatment. As mentioned briefly in regard to fig. 2, addition of neurocan to the culture solution was seen to preferentially deposit on the surface of the nanofiber substrate shortly after being added to coating solutions. This coating is shown to repel neurite-nanofiber attachment at lower fiber density.

25 Conclusion

These data show that when the fibers are coated with HSPG nerve outgrowth is stimulated in vitro. In vivo this is not considered essential due to infiltration of supporting cells, such as schwann cells and fibroblasts, that serve to coat the implant with extracellular matrix molecules.

30

Example 4 - 3D Nanofibrous Conduits

The translation of 2-dimensional nanofibers into a 3-dimensional aligned electrospun nanofiber conduit required an inventive step to facilitate the delicate insertion of nanofiber bundles of silk fibroin inside a hollow PCL tube (Fig. 5a). The conduit total length is 1.5 cm (Fig. 5b), and lumen diameter is measured at ~ 1.6

mm with a tube wall thickness of 200 μm . Upon insertion into the tube, the fibers were clearly aligned (Fig. 5c and 5d). Once the nanofiber surface treatment was completed, and coatings and fibrous 3D structure were stabilized by freeze drying. Imaging after lyophilization, shows the fibers clumped together due to their
5 respective coatings, and the addition of sucrose (Fig. 5c and 5e). When re-hydrated for implantation, the hydrophilic nanofibers expand and fill the void (figures 6 and 7).

Conclusion

10 These data shows that it is possible to construct 3D conduits according to the present invention.

Example 5 – in vivo rat test

Material and Methods:

15 Preparation of Nanofibrous Conduits

By density analysis, a defined amount of nanofibers from the collection surface was gathered and wrapped in a band around the tips of a 50 mm wide winder made from a 1.0 mm stainless steel rod. Stationary fibres suspended between winder prongs were then sequentially treated in PDL and laminin with or without
20 heparan sulphate proteoglycans, with the final washes containing 100 mM sucrose to stabilise the proteins for freeze-drying and storage. The suspended fibre bundle on the winder was threaded through the PCL tubes by looping a double butted PCL fiber around the middle of the bundle and then inserting the thick end into the tube. The thicker section of PCL fibre is stiff enough to be pushed through the
25 whole length of the tube without piercing or destroying the lumen like a needle. The thinner midsection of the fiber allows for pulling relatively thick bundles of silk nanofibers through. The PCL fibre holding the nanofibers was severed once pulled to the opposite end of the tube. The resulting tubes containing the fibres were placed in an eppendorf tube, snap frozen in a dry ice and ethanol bath, and freeze
30 dried. Before use in animals, the tube was freeze-cleaved in liquid nitrogen at the required length for surgical manipulations, and was rehydrated in saline solution.

Implantation of Conduits

Two groups of 3 animals were used to assess the effect of coating the conduits
35 with HSPG. Conduits were each implanted into 2mm long Sprague Dawley rat C3–

C4 spinal cord hemisections. The lesion included the lateral funiculus as well as the adjacent grey matter.

Implantation into Spinal Hemisections

- 5 Preliminary findings indicate ingrowth of raphespinal 5HT-positive and sensory CGRP-positive axons into the conduits.

Conclusion

- Our findings indicate that the invention is functional for the purpose of
10 regenerating spinal cord tissue in vivo.

Discussion of results:

- Patients with spinal cord injuries can expect limited recovery during the first 9 – 12 months if at all, which indicates a level of neuronal plasticity available for
15 surgeons to take advantage of, should an appropriate device be available. Guiding regenerating neurites/axons across injury sites is essential for the development of conduit devices for spinal nerve and long gap peripheral nerve defects. While guidance is important to increase functional reconnection of severed axon tracts, the speed at which this regeneration happens is of utmost importance, as it's
20 been shown that astrocytes begin depositing CSPGs within 24h after injury, and high concentrations persist in the injury site for months.

- Without aligned fiber guidance, nerve regeneration results do not quite compare to that of autograft controls. Whereas the best performance from an electrospun
25 conduit for peripheral nerve regeneration is a thin film of aligned fibers rolled into a tube and sealed (e.g. WO 2007/089 259). It is also worth noting that the dense packing of the aligned fibers, by film deposition and rolling, cannot support ingrowth inside the sheets, instead, relying on the material to degrade for ingrowth to occur. By comparison, the non-woven electrospun nanofibers
30 presented here can allow for far more ingrowth due to the increase surface area of guiding nanofibers.

Claims

1. A conduit (1) for regeneration of biological material, said conduit comprising
- a first material (2), having a through-going hole (3);
 - a plurality of fibers (6);
- 5 ○ the fibers (6) being aligned along the long-axis in the through-going hole (3);
- the fibers (6) having a maximum cross-sectional width in the range 200-2000 nm, and
- 10 ○ the fibers (6) fill 5-90% (by volume) of the through-going hole (3) of the first material (2).
2. The conduit according to claim 1, wherein said fibers (6) are distributed evenly or substantially evenly throughout the through-going hole (3) of the first material (2).
- 15 3. The conduit according to any of the preceding claims, wherein the first material (2) comprises PCL, Polyglycolic Acid (PGA), PLA (PLLA), PLGA, peptide amphiphile solutions, PEG, PLA-b-PEG-b-PLA, collagen, elastin, chitosan, ϵ -caprolactone and ethyl ethylene phosphate (PCLEEP), poly(ethylene-co-vinyl acetate) (PEVA),
- 20 crosslinked gelatin, fibrin, polydioxanone (PDO), PCL+PLA as copolymers, block copolymers or blends, silk, or combinations thereof.
4. The conduit according to any of the preceding claims, wherein the fibers comprises silk, silk fibroin, Polyglycolic Acid (PGA), PLLA, PCL, Peptide amphiphile
- 25 solutions, or mixtures thereof.
5. The fibers according to claim 4, wherein said mixtures thereof are fibers with one fiber encapsulating another fiber.
- 30 6. The conduit according to any of the preceding claims, wherein the fibers are in the form of fiber ribbons, round or oval, preferably round.

7. The conduit according to any of the preceding claims, wherein said first material comprises PCL and the fibers comprises silk fibers, or the first material comprises collagen and the second material comprises silk, or, the first material comprises PCL and the fibers comprises PGA, or the first material comprises PGA
5 and the fibers comprises PGA, or the first material comprises PCL and the fibers comprises PCL fibers, or the first material comprises PGA and the fibers comprises silk fibers, or the first material comprises silk and the fibers comprises silk fibers.

8. The conduit according to any of the preceding claims, wherein the fibers are
10 not silk fibers.

9. The conduit according to any of the preceding claims, wherein the silk is synthetic or recombinant silk or natural silk, such as silk from mulberry silkworm silk, non-mulberry silkworm silk, spider dragline silk, and/or bee silk.
15

10. The conduit according to any of the preceding claims, wherein the fibers are made of silk fibroin.

11. The conduit according to any of the preceding claims, wherein the conduit is
20 biodegradable and/or bio-absorbable.

12. The conduit according to any of the preceding claims, wherein the inner width of the hole is in the range 0.5 mm to 1 cm, such as in the range 0.5 mm to 6 mm, such as in the range 0.5 mm to 4 mm, such as in the range 0.5 mm to 2 mm,
25 such as in the range 1 mm to 8 mm, such as in the range 2 mm to 8 mm, or such as in the range 3 mm to 6 mm.

13. The conduit according to any of the preceding claims, wherein the outer width of the conduit is in the range 0.8 mm to 20 mm, such as in the range 1 mm to 20
30 mm, such as in the range 5 mm to 20 mm, such as in the range 10 mm to 20 mm.

14. The conduit according to any of the preceding claims, having a length in the range 5 mm to 10 cm, such as 1-10 cm, such as 3-10 cm, such as 5-10 cm, such
35 as 1-7 cm, such as 1-5 cm, or such as 1-3 cm.

15. The conduit according to any of the preceding claims, wherein the maximum diameter of the fibers is in the range 200-800 nm, such as 300-800 nm, such as 400-800 nm, such as 500-800 nm, such as 200-600 nm or such as 400-600 nm,
5 or such as 700-900 nm.

16. The conduit according to any of the preceding claims, wherein at least some of the fibers protrude from at least one end of the first material, such as both ends, with a distance of 0.1 mm to 1 cm, such as 2 mm to 1 cm, such as 5 mm 1
10 cm.

17. The conduit according to any of the preceding claims, wherein the number of fibers in the inner void is in the range $10^3 - 10^8$, $10^5 - 10^8$, or such as $10^6 - 10^8$.

15 18. The conduit according to any of the preceding claims, wherein said fibers fill 20-80% (by volume) of the inner void, such as 30-80%, such as 40-80%, such as 40-70%, such as 40-60%, or such as 60-80% of the inner void.

19. The conduit according to any of the preceding claims, wherein said conduit is
20 tube-shaped, oval, round, or polygonal.

20. The conduit according to any of the preceding claims, wherein the inner and/or outer side of the first material is smooth or substantially smooth.

25 21. The conduit according to any of the preceding claims, comprising one or more biologically active substances.

22. The conduit according to any of the preceding claims, wherein the biologically active substances are selected from the group consisting of growth factors,
30 cytokines, antibiotics, immunosuppressants, steroids, hormones, non-steroidal anti-inflammatory drugs (NSAIDs), peptide sequences such as RGD, acrylic acid, nucleotides, small interfering RNAs, antisense RNA, and/or heparin sulfate proteoglycans.

23. The conduit according to any of the preceding claims, wherein the fibers or fraction of the fibers are coated with heparin sulfate proteoglycan (HSPG), laminin, and/or NGF (Nerve growth factor).

5 24. The conduit according to any of the preceding claims, wherein the biologically active substances are coated on the fibers, and/or on the outer surface of the first material and/or on the inner surface of the first material.

25. The conduit according to any of the preceding claims, further comprising living
10 cells, preferably in the through-going hole (3) in the first material.

26. The conduit according to claim 25, wherein the cells are nerve cells, such as Schwann cells and/or olfactory ensheathing glia (OEG), and/or adipose stems cells, and/or mesenchymal stem cells and/or induced pluripotent stem cells.
15

27. The conduit according to any of the preceding claims, wherein a portion of the fibers (6) are aligned in opposite directions, such as half of the fibers or wherein a portion of the fibers (6) are aligned at angle.

20 28. The conduit according to any of the preceding claims, wherein at least some of the fibers (6) are not in direct contact with the first material (2).

29. The conduit according to any of the preceding claims, wherein the first material (2) is not a rolled film or mat.
25

30. The conduit according to any of the preceding claims, wherein the conduit is for regeneration of biological material selected from the group consisting of muscles fibers, bone, tendons, and/or cartilage, preferably nerves.

30 31. The conduit according to any of the preceding claims, wherein the remaining void in first material is filled with a gel, and/or liquid such as a saline or nutrient.

32. The conduit according to any of the preceding claims, wherein the first material is porous.
35

33. The conduit according to any of the preceding claims, wherein the conduit is suitable for implantation in a mammal, such as a human.

34. The conduit according to any of the preceding claims, wherein the conduit is
5 sterile.

35. The conduit (1) according to any of the preceding claims, for use as a medicament.

10 36. The conduit (1) according to any of claims 1-34, for use in regeneration of nerves, muscles fibers, bone, tendons, or cartilage.

37. The conduit (1) according to claim 36, for use in nerve regeneration, such as regeneration of peripheral nerves and/or spinal nerves.

15

38. Use of the conduit (1) according to any of claims 1-34 for ex vivo growth of nerves, muscles fibers, bone, tendons, or cartilage.

39. Use according to claim 38, for nerve regeneration, such as peripheral nerves
20 and/or spinal nerves.

40. A process for producing a conduit (1) comprising

- 1) providing a first material (2) having a through-going hole (3);
 - 2) providing a plurality of fibers (6), the fibers fiber having maximum cross-sectional width in the range 200-2000 nm;
25
 - 3) threading said fibers through the through-going hole (3);
 - 4) optionally, freeze-drying the first material (2) comprising the fibers (6) distributed in the inner void;
 - 5) optionally cleaving said first material at a desired length,
30
- thereby providing a conduit comprising,
- a first material (2), having a through-going hole (3);
 - a plurality of fibers (6)
 - o the fibers (6) being aligned along the long-axis in the through-going hole (3);

- the fibers (6) having a maximum cross-sectional width in the range 200-2000 nm, and
- the fibers (6) fill 5-90% (by volume) of the through-going hole (3) of the first material (2).

5

41. The process according to claim 40, wherein the fibers (6) provided in step 2) are produced by electrospinning.

42. The process according to claim 41, wherein the fibers are produced by co-
10 spinning two or more solutions.

43. The process according to claim 41 or 42, wherein the electrospun fibers are collected on a rotating drum.

15 44. The process according to claim 43, further comprising collecting the electrospun fibers, thereby providing fibers free from the rotating drum.

45. The process according to claim 44, where the electrospun fibers are collected around two rods.

20

46. The process according to claim 41 or 42, wherein the electrospun fibers are deposited directly on a charged collection device comprising two rods, thereby providing fibers aligned parallel between the rods.

25 47. The process according to claim 45, wherein the charged collection device is positively or negatively charged.

48. The process according to any of claims 44-46 further comprising wrapping a tread around the fibers before step 3).

30

49. The process according to claim 48, wherein said thread wrapped around the fibers (6) is made of a polymer such as PCL.

50. The process according to any of claims 40-49, wherein during electrospinning the fiber is mixed with a viscosity-increasing agent such as polyethylene oxide (PEO).

5 51. The process according to any of claims 40-50, wherein the diameter of the fibers are adjusted by increasing or decreasing the distance between the emitter and collector devices.

52. A conduit obtainable/obtained by process according to any of claims 40-51.

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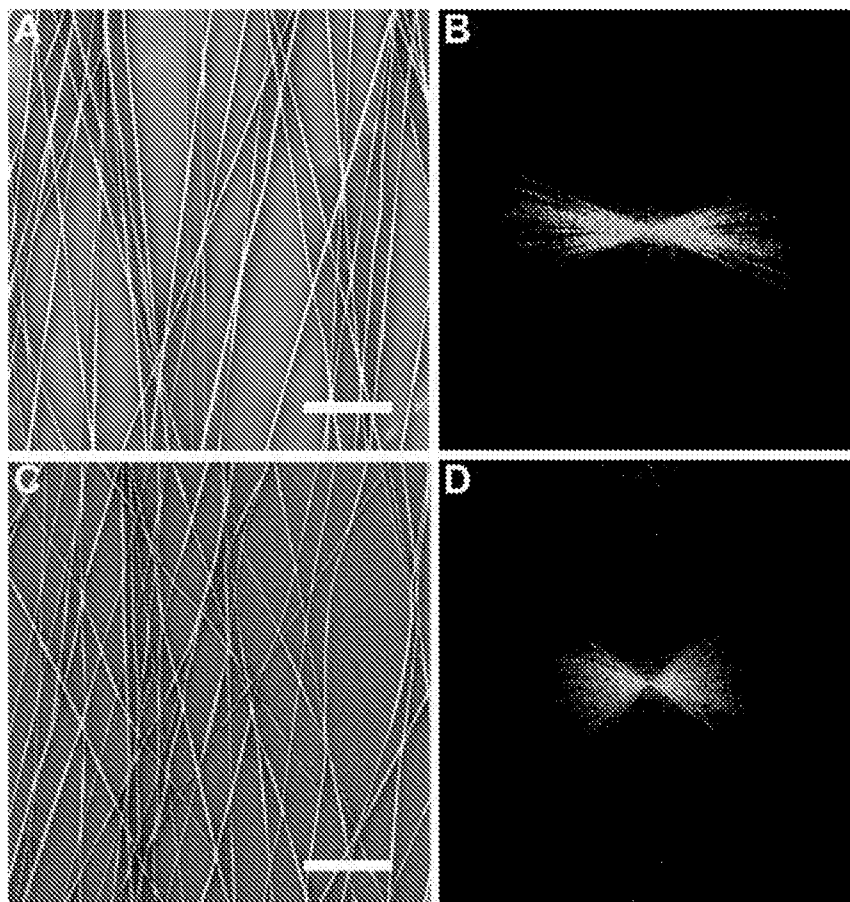


Fig. 1

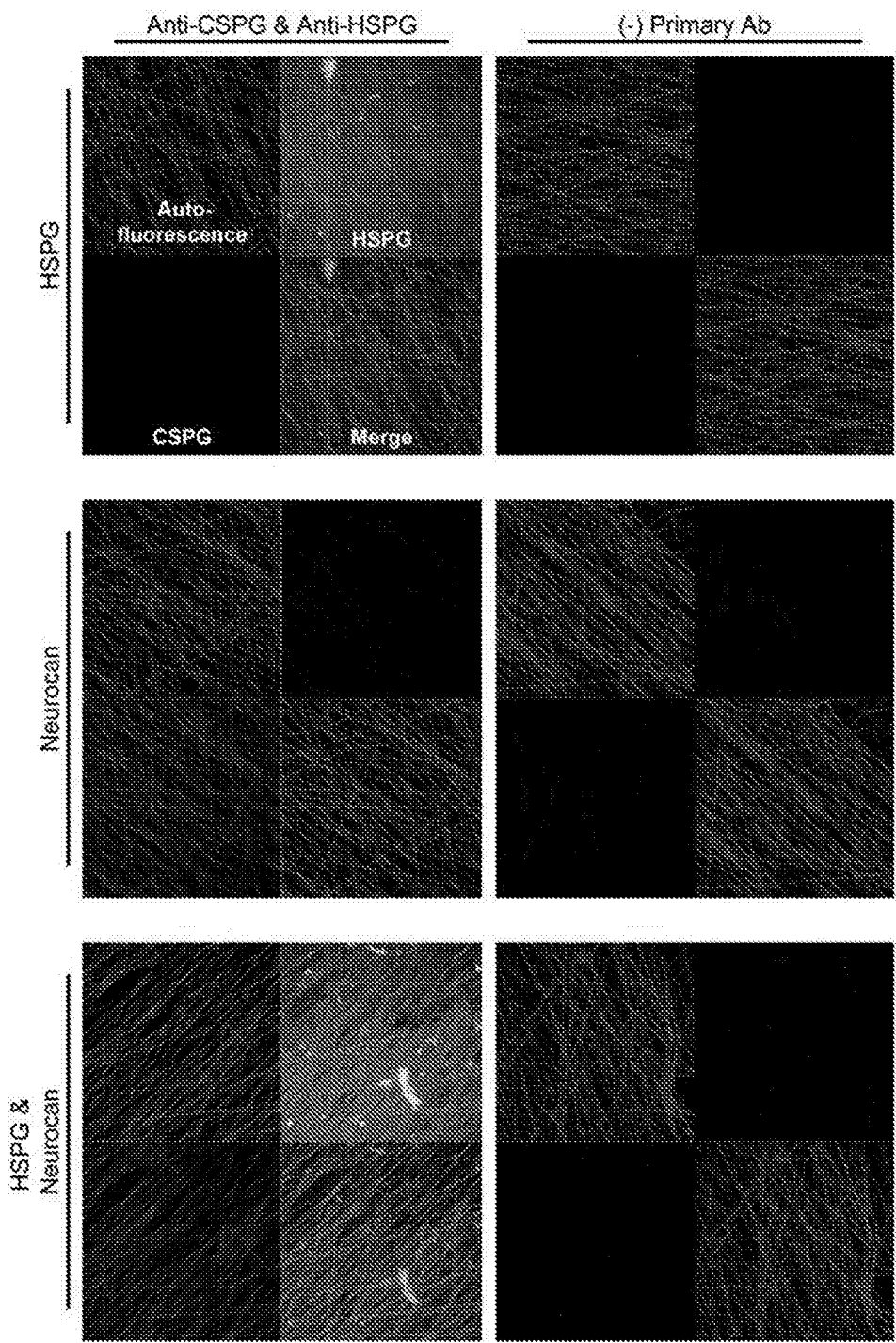


Fig. 2

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A

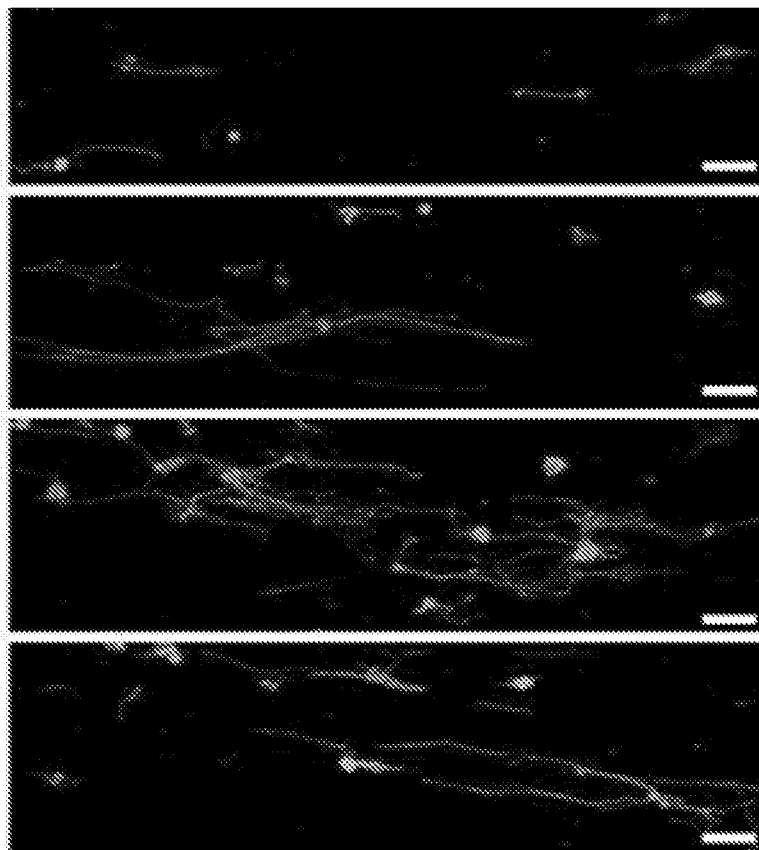


Fig. 3 - continues

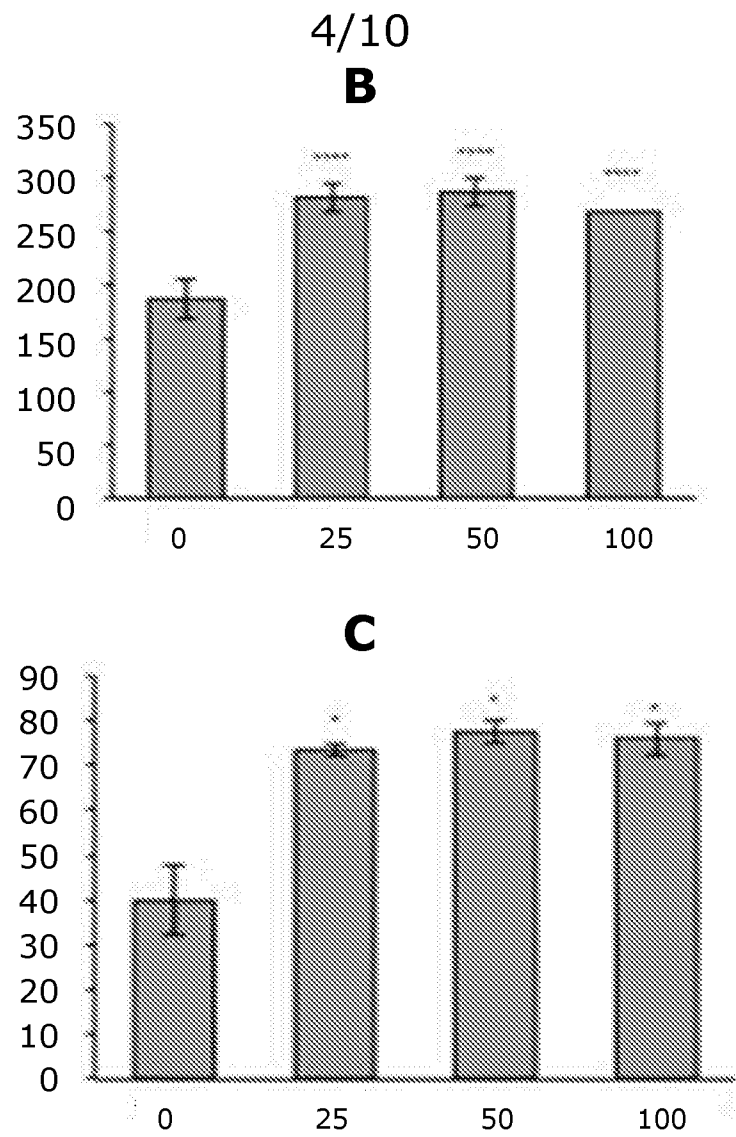


Fig. 3 - continued

(1)

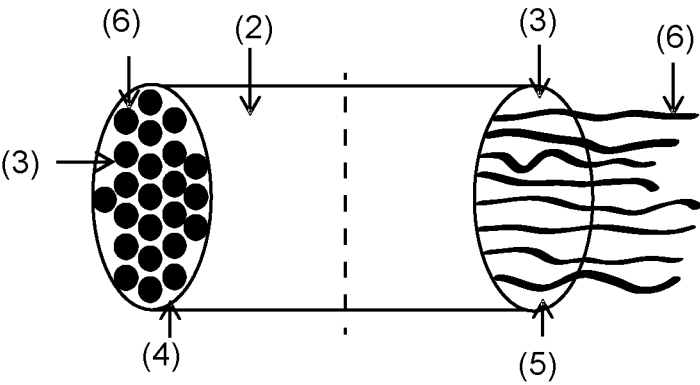


Fig. 4

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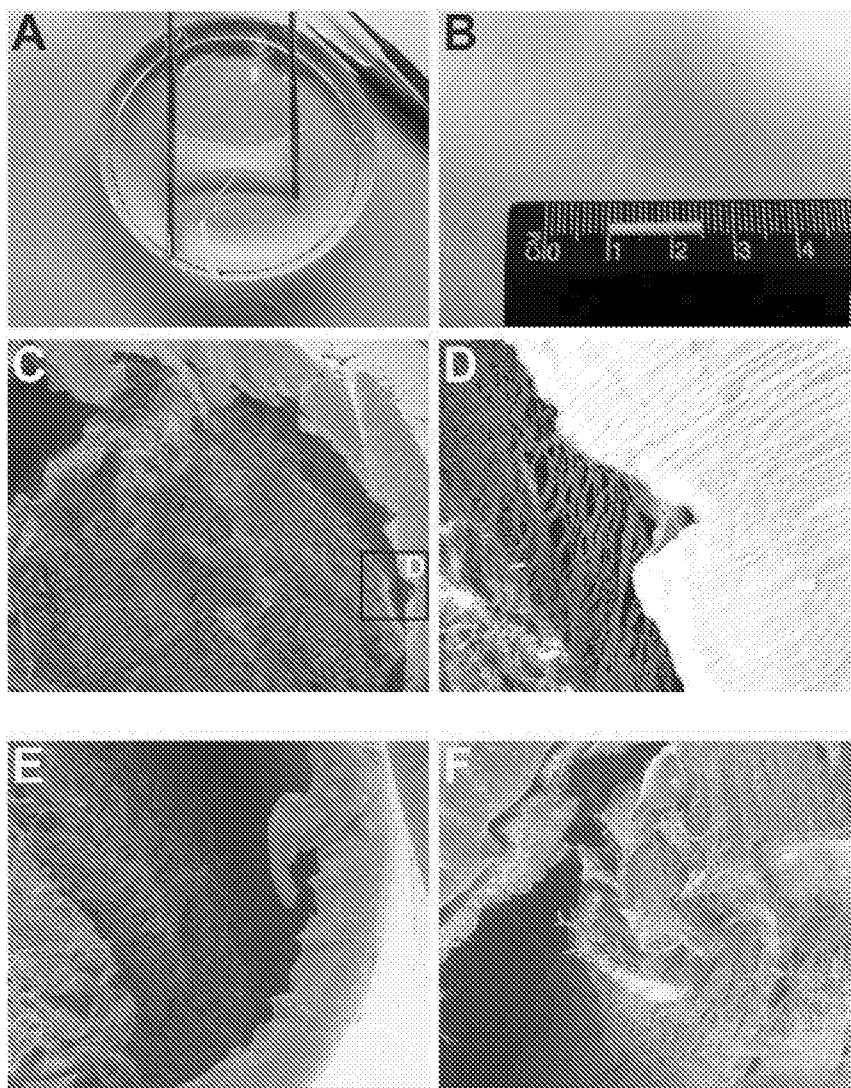


Fig. 5

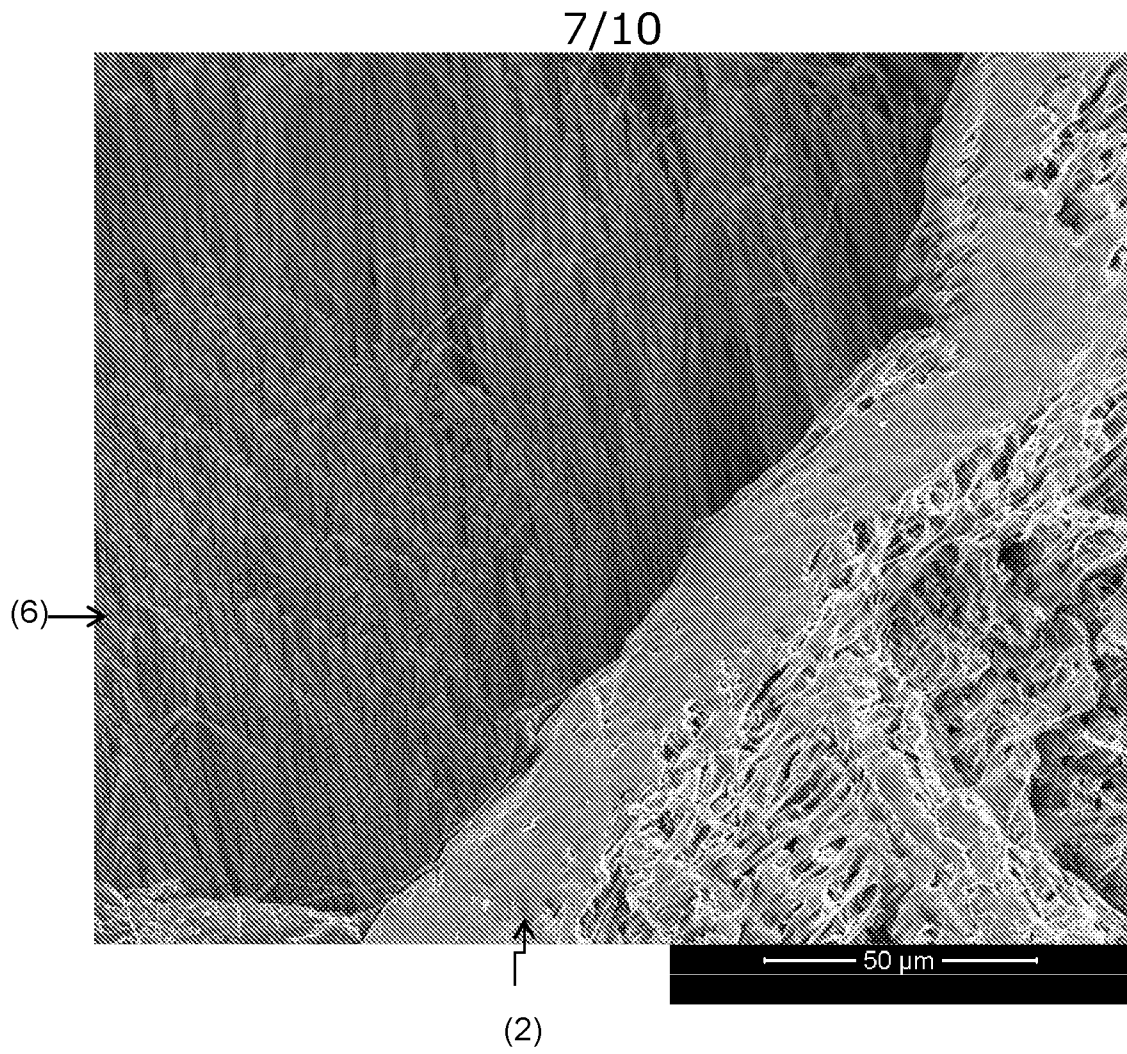


Fig. 6

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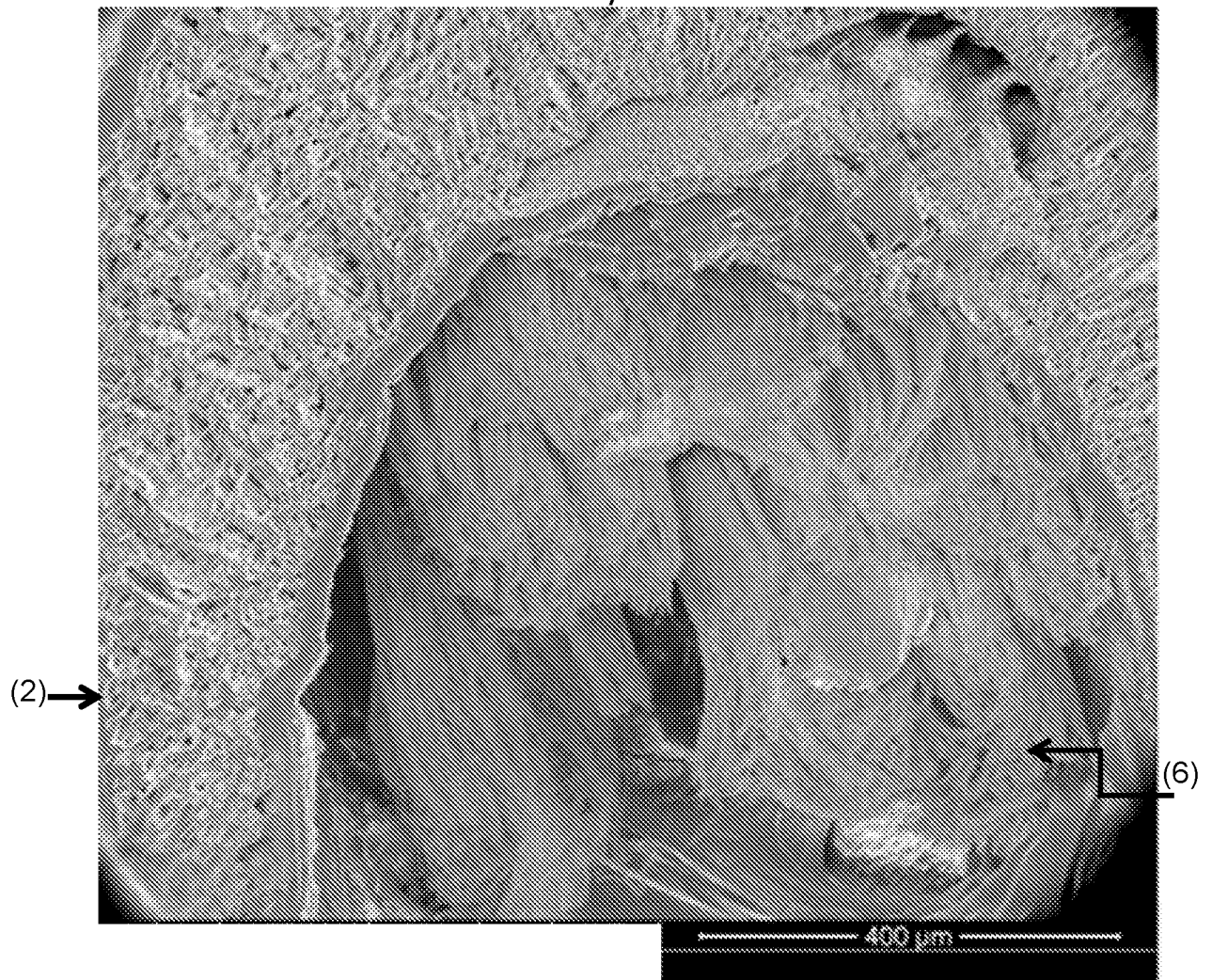


Fig. 7

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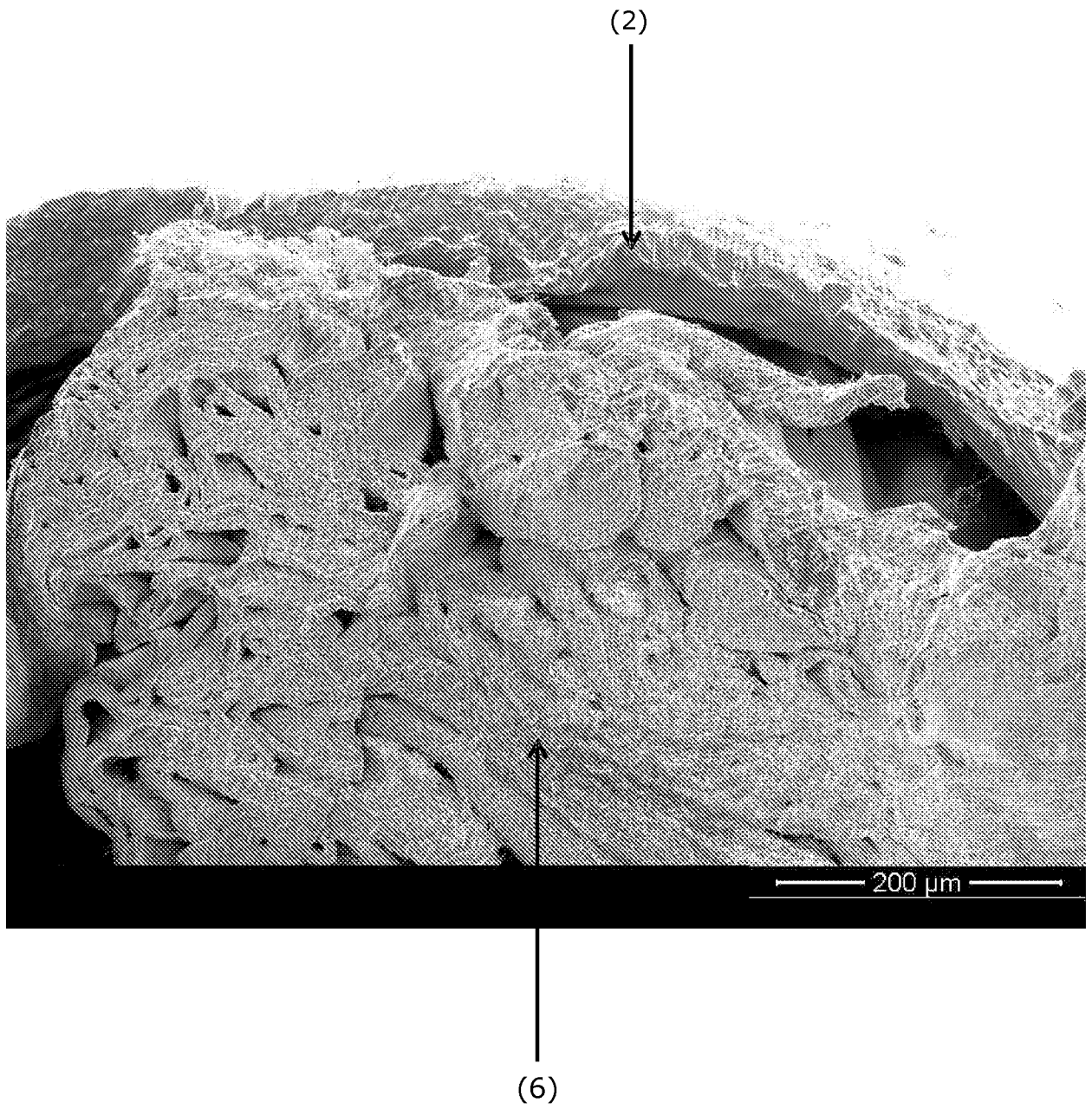
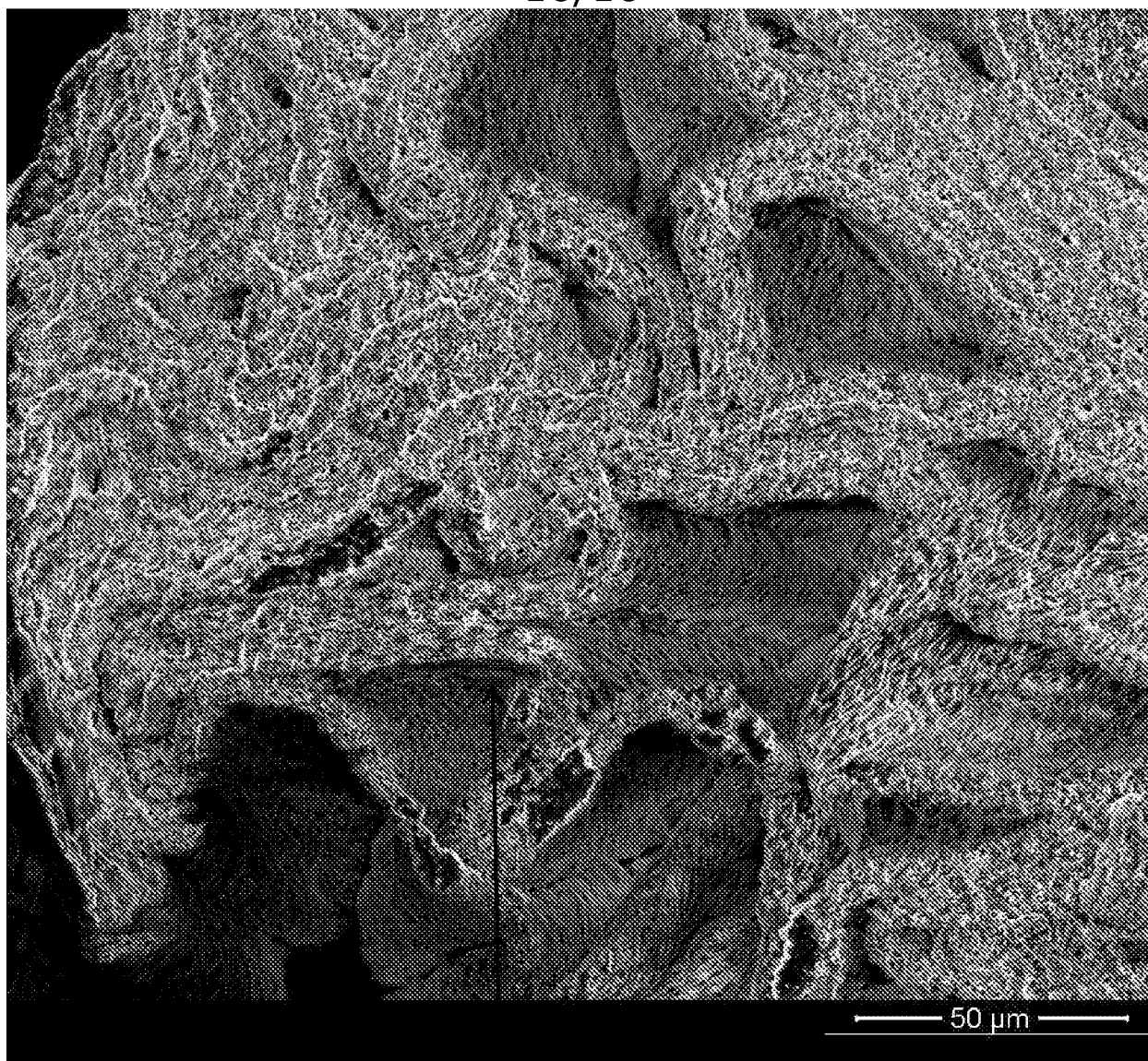


Fig. 8

10/10



(6)

Fig. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2016/050156

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61L27/22 A61L27/36 A61L27/38 A61L27/52 A61L27/54
A61L27/56 A61L27/58

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, BIOSIS, EMBASE, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/134352 A1 (UAB RESEARCH FOUNDATION [US]) 12 September 2013 (2013-09-12) claims 1-3, 9, 10,12, 17, 15 figure 3 page 6, paragraph 4 - page 7, paragraph 3 -----	1-52
X	WO 2010/062297 A1 (JACKSON H M FOUND MILITARY MED [US]; US ARMY [US]; US NAT INST OF HEAL) 3 June 2010 (2010-06-03) example 5 claims 1-28 figures 1-2 paragraphs [0083] - [0085] ----- -/--	1-52



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

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