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Title:

ELECTROSPINNING SYSTEM AND PROCESS FOR LARGE SCALE MANUFACTURING OF ALIGNED 3D FIBER MATRICES

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ABSTRACT

"ELECTROSPINNING SYSTEM AND PROCESS FOR LARGE-SCALE MANUFACTURING OF ALIGNED 3D FIBER MATRICES"

This invention relates to the system and process of continuous electrospinning for the production of three-dimensional matrices of aligned polymeric fibres.

The system of the present invention comprises an electrospinning capillary tube (3) with positive polarity, a set of multi-electrodes with negative polarity inserted in a peripheral support (15), having each electrode (7) controlled movement allowing its exposure or retraction-occultation to the electrospinning tube (3), a central collecting table (17) of electrospun fibres, which is covered with holes (6,16) connected to a chamber and to a vacuum pump (13).

The formation of aligned three-dimensional matrices (30) occurs by deposition of layers, when exposing the electrodes (7) to the capillary tube (3), with the controlled distancing of the central collecting table (17), with respect to the electrospinning tube (3).

The present invention has application in the medical field, in tissue engineering, in particular in regenerative medicine.

DESCRIPTION

" ELECTROSPINNING SYSTEM AND PROCESS FOR LARGE-SCALE MANUFACTURING OF ALIGNED 3D FIBER MATRICES"

Technical field of the invention

This invention relates to a continuous electrospinning system and process for the production of three-dimensional matrices of aligned polymeric fibres.

From the invention applicability, it is possible to obtain three-dimensional matrices of aligned polymeric fibres, which can present several patterns of fibre alignment along the matrix thickness, being this thickness dependent on the number of deposited fibre layers, the fibre thickness and the degree of compaction between layers.

In this way, the present invention has been applied in various areas, in the manufacture of products or structures, at the nanometric scale, which depend on the high surface area, such as biotechnology, pharmaceuticals, research, tissue engineering and medicine, particularly in regenerative medicine, such as cell therapy, cartilaginous and related tissue production, especially for the replacement and strengthening of joints.

Prior Art

Among the main themes of interest in the processing of polymeric materials is the production of micro or nanostructured polymeric structures, especially nanofibres or nanowires. The unique properties of nanomaterials associated with the different possibilities of morphologies and functionalities reveal a series of possibilities for new fields of application and drive the progress in the processing of these nanostructures.

In this regard, the electrospinning or electrostatic spinning method is very advantageous, since the fibres obtained with this technique have a high surface area, combined with a low production cost and the possibility of being formed from a wide variety of polymers or composites. This technique is based on the application of high voltage (5-50 KV) and low current (0.5-1 μA) electric fields for the production of very small diameter fibres. In this process, the electrostatic forces control the formation and deposition of these fibres.

The document US2349950A describes a basic experimental arrangement, in which the proposed diagram already presents a configuration formed by a high voltage source, polymeric solution and a grounding system.

Currently, the key configuration of a generic electroplating process consists of a syringe, where the molten polymer or polymeric solution is introduced, which is connected to a capillary tube, a diffuser pump, which controls the flow of the polymeric solution to be supplied, so that a drop of solution is always maintained at the tip of the capillary tube, a metal collector, maintained at zero potential (grounded), where the fibres produced will be collected, a high voltage source, responsible for producing a difference in potential between the tip of the capillary tube and the collector. With the application of the electric field between the capillary tube and the collector the drop of solution is subject to the orientation of loads on its surface.

As the field intensity increases, the balance of electrostatic charges to which the droplet is subjected, namely the surface tension force of the solution and the force exerted by the applied electric field, begins to suffer an imbalance and, from a certain critical value of electric field, a jet of polymeric material from the capillary tube is projected and accelerated towards the collector.

During the trajectory to the collector, the jet with the polymeric solution suffers evaporation of a large part of its solvent, thus ensuring that the fibres formed have enough rigidity to support their own weight. In addition, the solvent that remains in the solution, such as moisture, allows the adhesion of one fibre to another, as they are deposited in layers, forming a non-woven web. In this basic configuration, the electrospun fibres form a two-dimensional, randomly oriented blanket or fabric due to the instability of the jet path.

Interest in the electrospinning process has grown very rapidly since the 1990s. Multidisciplinary efforts, both in the area of academic and application-oriented research, have generated a huge number of scientific publications, patent applications and a significant increase in the exploitation of the technique by companies of filtration products, regenerative medicine, protective clothing, catalysis, among others.

Oriented fibre networks have the possibility of developing anisotropic properties in materials. These relationships are quite obvious in the field of tissue engineering.

Typical examples include the production of polymeric meshes, containing aligned fibres, used as substrates for culture and regeneration of neural cells, due to the inherently anisotropic nature of nerves and their regenerative mechanisms. In these bioengineering applications, it is a fundamental requirement that the scaffold material has a three-dimensional structure of controlled porosity, so that it is possible to develop three-dimensional cell construction at the full depth of the matrix.

Many efforts have been concentrated on the production of aligned fibres with controlled standardization, due to its exceptional potential for development of functional devices, such as those presented in documents US20120009292A1, US20110142806A1 and US2016004706A1.

Several approaches have been suggested to promote the alignment of the electrospun fibre blankets, among which the "air gap electrospinning" process, which foresees the configuration of spaced parallel electrodes, with the fibres stretched in the spacing between the plates, has been the most used method to deposit and collect these fibres.

The publication of a pioneering work by Dan Li and collaborators (Li, et al., Nanoletters, 2003, 3:8, 1167) showed that two effects favoured the production and deposition of nanofibres well aligned between the electrode space parallel to each other, namely the effect of nanofibres deposition direction, caused by deformation of the electric field between the capillary tube and the collector, and the accumulation of charges along deposited nanofibres, which favoured the parallel arrangement between them, due to electrostatic repulsion.

An interesting variation of this assembly system is the collector for production of fibre matrices, whose system comprises electrodes arranged in a 90° separated plane. The operation is based on the connection of the ground terminal to the electrodes arranged in the same line. The electrospun fibres are collected between the electrodes, which are connected to ground, and this connection is alternated between the pairs of electrodes with defined time intervals, allowing the formation of a mesh with layers of fibres with different arrangements (Li, et al., Adv. Matter, 2004, 16:4, 361).

In this regard, document US20110018174A1 discloses the production of aligned electrospun fibres, with location and orientation control of the fibres, using for this purpose a device that provides a voltage depending on the selected time, whereby that voltage is applied to a collector with multiple electrodes. However, said document does not disclose a process capable of forming a three-dimensional matrix of aligned fibres in any desired thickness.

Other strategies for the formation of three-dimensional matrices of aligned fibres have been the subject of studies, mainly in the field of regenerative medicine, such as, for example, the articles: Sheikh, et.al., Nanomedicine, 2015, 11, and Li, et.al., Mater. Sci. Eng. C.,2016,68.

In this regard, the document US 8580181B1 also discloses a method of forming three-dimensional matrices of nanofibres aligned with an open and loose structure.

Although the configurations above consider the formation of multiple fibres layers aligned one over the other, in the space between the electrodes, there are still some problems with the formation of three-dimensional matrices. The limitations related to the current processes of electrospinning the aligned fibres are mainly related to the fact that, as the aligned and electrically charged fibres are deposited one over the other, the increasing electric charge tends to repel the new fibres from being deposited, preventing their correct alignment and limiting their thickness to a few tenths of a millimetre of the matrix of the formed fibres.

For certain purposes, such as tissue engineering, the formation of high thickness fibre matrices in the order of several millimetres is necessary, with control of the fibre alignment along the thickness and with the possibility to control the degree of compaction (porosity) between the deposited aligned fibre layers.

This way, there is a need to develop and implement a process of production of three-dimensional matrices of aligned polymeric fibres, which allows the production of several patterns of fibre alignment, along the thickness of the matrix, forming three-dimensional structures with controlled thicknesses.

The present invention proposes to solve the problems of the state of the art above described, through the implementation of a system and process of production of three-dimensional matrices of aligned polymeric fibres, which can present several patterns of fibre alignment, along the thickness of the matrix, being this thickness dependent on the number of layers of deposited fibres, the thickness of fibres and the degree of compaction between layers.

Summary of the invention

This invention relates to a continuous electrospinning system and process for the production of three-dimensional matrices of aligned polymeric fibres.

The formation of three-dimensional matrices of aligned polymeric fibres occurs when one or more pairs of electrodes are exposed to the electrospinning capillary tube, thereby creating a layer of two-dimensional aligned fibres, on which are successively deposited, other layers formed by exposure of one or more pairs of electrodes to the capillary tube, accompanied, subsequently, by controlled movement away from the central collecting table of the capillary tube, after each layer of deposited fibres, in accordance with the claim 1.

Thus, the process of the present invention allows the production of three-dimensional matrices of aligned polymeric fibres, which can present several patterns of fibre alignment along the thickness of the matrix, being this thickness dependent on the number of deposited fibre layers, fibre thickness and the degree of compaction between layers, in accordance with the claim 7.

The produced matrices are used in various areas, such as biotechnology, pharmaceuticals, research, tissue engineering and medicine, in particular regenerative medicine, such as cell therapy and the production of cartilaginous and related tissue, in particular for joint replacement and strengthening, in accordance with the claim 9.

Additionally, the process of the present invention, by using the proposed electrospinning system, has the additional advantage of being versatile, simple, inexpensive and working in a continuous mode, therefore not being necessary to produce series of layers with a certain alignment and to proceed to add other layers, with different alignment, to obtain three-dimensional matrices of polymeric fibres aligned with different patterns of fibre alignment and different thicknesses.

The system of the present invention comprises an electrospinning capillary tube, a set of multi-electrodes, in which each electrode presents a controlled movement of exposure or retraction- occultation in relation to the electrospinning tube, and a central collecting table of the electrospun fibres, which comprises holes subject to vacuum pressure, with the possibility of regulating the distance between the electrospinning capillary tube and the electrodes in exposure position, and with controlled movement, towards the capillary tube axis, in accordance with the claim 12.

The system of the present invention, through the controlled movement of exposure or retraction-occultation of the electrodes, in relation to the electrospinning tube, with controlled vacuum production applied to the holes of the central collecting table, allows to secure the fibres to the table, as well as to achieve a certain degree of compaction (and consequently porosity in vertical direction) necessary for certain uses of the fibres. In addition, the controlled downward movement of the table allows the formation of three-dimensional aligned fibre structures.

Brief description of the drawings

Figure 1: Schematic depiction of an embodiment of an electrospinning system, according to the present invention, with two exposed electrodes, the following numbers representing:

1 electrospinning system;
2 electrospun fibre;
3 electrospinning capillary tube;
4 positive polarity connection between the electrospinning capillary tube (3) and a power supply (not shown);
5 capillary tube bracket (3), resting on the mounting base (8);
6 holes in the peripheral support, where the multi-electrodes are inserted (7,14);
7 electrode in exposure position, with respect to the electrospinning capillary tube (3) with negative polarity;
8 fixing base for the entire electrospinning system (1);
9 computer control unit for the entire electrospinning system (1);
10 induction coil for generating the electromagnetic force for the electrospinning system (1);
11 permanent magnet attached to an electrode (7,14);
12 linear motion actuator of the central collecting table (17);
13 vacuum pump with pressure regulation;
14 electrode in retraction-occultation position, with respect to the electrospinning capillary tube (3);
15 peripheral support of the collecting table (17), made of insulating material;
16 holes in the surface of the central collecting table (17), which connect to the inside of the chamber (not shown);
17 central collecting table; and

18 connecting channel between the inner chamber (not shown) of the central collecting table (17) and the vacuum pump (13).

Figure 2: Schematic depiction of a longitudinal section of the electrospinning system (1), according to a preferred embodiment of the present invention, in which:

- The electrospun fibre (2) is located between the two electrodes exposed with negative polarity (7) and the collecting table (17) with its surface (19) covered with holes (16) connected to the chamber (21) inside it, which is submitted to vacuum pressure, in the position closest to the electrospinning capillary tube (3);
- Two electrodes are in exposure position (7) on the surface (20) of the peripheral support (15), one electrode is in a concealed position (14);
- the actuator (12) of the linear movement (24) of the collecting table (17),
- the senses the electromagnetic forces of attraction (23) and repulsion (22) generated between the end of the electrodes (7)(14) with permanent magnet (11) and the electromagnetic coils (10);

Figure 3: Schematic depiction of a longitudinal section of the electrospinning system (1), according to a preferred embodiment of the present invention, in which:

- the electrospun fibre (2) is deposited between the two exposed electrodes (7) and the central collecting table (17), covered with holes (16), connected to the chamber (21), inside it, in a position further away from the electrospinning capillary tube (3);
- the direction of suction force (25), fixation-compaction of the different fibre layers (27) (28) (29), generated by the

effect of vacuum pressure in the chamber (21), and the thickness (26) of the fibre matrix formed after the deposition of different layers (27) (28) (29) of aligned fibres.

Figure 4: Schematic depiction of one of embodiments used in the invention's electrospinning process, in which:

- a first layer of aligned fibres (30) lies between two exposed electrodes (7) with negative polarity, belonging to the multi-electrode array, which are in a concealed position in the holes (6) of the peripheral support (15), with the central collecting table (17) in the closest operating position to the electrospinning capillary tube (3);
- the central collecting table (17) through its chamber (21) connected to it.

Figure 5: Schematic depiction of the aligned fibres of the first layer (30) fixed to the surface (19) of the central collecting table (17), by action of the suction force (25) generated in the holes (16) of the surface (19) of the central collecting table (17), by effect of the vacuum present in the chamber (21) of the central collecting table (17), which, after a certain period of time, moved away from the electrospinning capillary tube (3), by the action of the movement (32) of the actuator (12), and **is followed by the deposition of the second layer of aligned fibres** (31), between the two exposed electrodes (7) and by the movement away from the table (32).

Figure 6: Schematic depiction of the aligned fibres of the first layer (30) and second layer (31) fixed on the surface (19) of the central collecting table (17), by action of the suction force (25) generated in the holes (16) of the surface (19) of the central collecting table (17), by the effect of

the vacuum present in the chamber (21) of the central collecting table (17), which, after a certain period of time, moved away from the electrospinning capillary tube (3), by action of the movement (33) of the actuator (12), and **is followed by the deposition of the third layer of aligned fibres** (31), between the two exposed electrodes (7).

Figure 7: Schematic depiction of the aligned fibres of the **first layer (30)**, **second layer (31) and third layer (32)**, fixed on the surface (19) of the central collecting table (17), by the action of suction force (25) generated in the holes (16) of the surface (19) of the central collecting table (17), by the effect of vacuum pressure, present in the chamber (21) of the central collecting table (17), which, after a certain period of time, moved away from the electrospinning capillary tube (3), by action of the movement (36) of the actuator (12), and **is followed by deposition of the fourth layer of aligned fibres (35)** between the two exposed electrodes (7);

Figure 8: Schematic depiction of the top view of the central collecting table (17) and peripheral electrode support (15) **with the representation of nine fibre alignments** (301) (302) (303) (304) (305) (306) (307) (308) (309), **deposited sequentially in layers**, being the alignment (301) obtained by exposing the electrodes (702) and (710), the alignment (302) obtained by exposing the electrodes (702) and (705), the alignment (303) obtained by exposing the electrodes (701) and (706), the alignment (304) obtained by exposing the electrodes (701) and (706), the alignment (305) obtained by exposing the electrodes (708) and (702), the alignment (306) obtained by exposing the electrodes (706) and (711), the alignment (307) obtained by exposing the electrodes (710) and (707), the alignment (308) obtained by exposing the electrodes (709) and

(702), the alignment (309) obtained by exposing the electrodes (710) and (703), in which the set of these nine layers forms an alignment pattern (200), which is repeated successively, between the first (203), intermediate (205) and last (206) pattern, thus resulting in the thickness (26) of the three-dimensional matrix, which is delimited by the dimensions (202) and (201).

Description of the invention

This invention relates to a system and a continuous electrospinning process for the production of three-dimensional matrices of aligned polymeric fibres, in particular for tissue engineering, with the possibility of producing several patterns of fibre alignment along the thickness of the matrix forming three-dimensional structures with controlled thicknesses.

The system of the present invention comprises a module of fibre formation, which can consist basically of a syringe to contain a polymeric solution, connected to an injection pump, connected to a electrospinning capillary tube, which is connected to a voltage source, configured to provide positive polarity, the referred module is aligned longitudinally with the peripheral support in insulating material in which they are inserted longitudinally in holes in its multi-electrode surface, each electrode being provided with individual movement controlled towards the axis of the electrospinning capillary tube, with two positions, an exposure position and a retraction-occultation position with respect to the electrospinning capillary tube, as well as the possibility of selective activation of the negative polarities of these electrodes, when these electrodes are in the exposed position with respect to the electrospinning capillary tube, the alignment and distribution of these electrodes in the peripheral support delimit the area and shape of the central fibre collecting table.

The fibre of polymeric material, formed by electrospinning from the capillary tube with positive polarity, moves by the action of an electric field towards a collector module, which consists of a peripheral support in insulating material where are inserted longitudinally multi-electrodes and each electrode equipped with controlled movement towards the axis of the electrospinning capillary tube allowing its exposure or retraction-occultation with respect to the electrospinning capillary tube, a central collecting table delimited by the peripheral support of the multi-electrodes which consists of the region of accumulation of the electrospun fibres integrating these holes which extend from its upper surface to the inside of the chamber being this chamber connected to a vacuum pump with pressure control, this central collecting table has controlled movement towards the axis of the capillary tube allowing its separation or approach to the electrospinning capillary tube.

The peripheral support of the multi-electrodes and the central collecting table are mounted on a fixed platform where the length-adjustable bracket of the electrospinning capillary tube is placed, allowing the distance between the electrospinning capillary tube and the multi-electrodes to be adjusted.

The exposure and retraction-occultation movement of the electrodes inserted into holes in the peripheral support to the electrospinning capillary tube results from a controlled electromagnetic force that develops at the end of the opposite electrode the upper surface of the peripheral support by the action of magnetic flux between a permanent magnet attached to the electrode and a fixed induction coil in the lower region of the peripheral support.

Fibre flow is deposited and aligned when one or more electrode pairs are held in the exposure position with respect to the electrospinning capillary tube, and their respective negative polarities are activated, according to the intended fibre deposition orientation, thus forming an aligned two-dimensional fibre layer.

The retraction-occultation movement of the electrodes, with respect to the electrospinning capillary tube, to the inside of its hole in the peripheral support of the multi-electrodes allows the deposit of the fibres on the central collecting table followed by the separation of the fibres from the end of the electrode.

The controlled movement away from the upper surface of the central collecting table of the electrospinning capillary tube, after each two-dimensional layer of aligned fibres has been deposited, allows the accumulation of successive fibre layers on the central collecting table thus allowing the formation of a three-dimensional fibre matrix structure in which its thickness is dependent on the number of layers of two-dimensional deposited fibres, the fibre thickness and the degree of intended compaction between layers by the vacuum system action.

The successive two-dimensional layers of deposited fibres in the collector module, according to the referred electrospinning process, are kept in position in the central collecting table, between the multi-electrodes, by the action of vacuum generated in the holes of the upper surface of the central collecting table that communicate with a chamber connected to the vacuum pump inside it.

The pressure control in the vacuum pump also has the purpose of controlling the degree of compaction between the two-dimensional fibre layers formed and so the porosity in the perpendicular direction to the plane of the deposited fibre layer.

Control of the distance between the electrospinning capillary tube with positive polarity and the collector module, control of the exposure and retraction-occultation movements of the electrodes with negative polarity with regard to the electrospinning capillary tube, control of the movement of the upper surface of the central collecting table in reference to the electrospinning capillary tube, control of the negative polarity applied to the multi-electrodes and the control of the vacuum pump pressure are carried out by a computerised control unit which, depending on the fibre alignment intended for each two-dimensional layer deposited and the thickness of the matrix, program the sequence of all the movements, vacuum pressure and polarity of the necessary electrodes based on a computer program developed for that purpose.

This invention has the ability to form matrices of aligned fibres of any thickness in continuous and with any alignment pattern along that thickness, because it combines different technical particularities, among them we highlight the use of multi-electrodes distributed spatially with the possibility of individual and controlled movements of exposure and retraction-occultation to the electrospinning capillary tube, thus allowing the possibility of controlling the alignment of deposited fibres, the support and deposit of the fibres on the central collecting table and the separation of the fibres from the ends of the electrodes during the retraction movement, the surface of the collecting table being provided with holes subject to vacuum pressure, which on one hand allow the fibres to be fixed to the table and also allow the control of the degree of compaction (porosity) between the different layers of deposited fibres, this capacity being also associated with the movement of the collecting table away from the electrospinning capillary tube, thus allowing the successive deposit of aligned fibre layers to the intended thickness.

1. Electrospinning system for the production of three-dimensional matrices of aligned polymeric fibres

The system of the present invention comprises, in a broad sense:

- a **fibre-forming module** comprising a container for the containment and supply of molten polymer or polymeric solution, typically a syringe, and an injecting pump, connected to an electrospinning capillary tube, connected to a voltage source, which is configured to provide positive polarity;
- a **multi-electrode assembly** comprising several electrodes with negative polarity;
- a **collector module** for collecting the produced fibres;
- a power supply to the electrospinning system; and
- a vacuum pump.

In addition to the aforementioned elements, the system of the present invention also comprises a permanent magnet, attached to each of the electrodes, to generate the forces of attraction and repulsion between the ends of the electrodes and the electromagnetic coils, also present in the system, a computerized control unit and the electronics necessary for its proper operation, actuators, including the actuator of the linear movement of the central collecting table, as well as all the electrical wiring for distribution of energy to the various components of the system.

More specifically, the system (1) of this invention comprises:

- a fibre-forming module with an **electrospinning capillary tube** (3);
- a **set of multi-electrodes**;
- a **collector module** to collect the produced fibres;
- a vacuum pump (13) for pressure regulation;

Wherein:

a) **The set of multi-electrodes**, which is inserted in a peripheral support (15), comprises:
  (i) multiple electrodes (7) (14), each with controlled movement, towards the axis of the electrospinning capillary tube (3);
  (ii) several magnets (11), each of which is attached to an electrode (7,14) for generating electromagnetic force (22, 23), together with the induction coil (10), controlled by the computer unit (9);

b) The **collector module** comprises:
  (i) a peripheral support (15), where the electrodes (7) (14) are inserted longitudinally into holes (6) on its surface (20), with the support (15) mounted on a fixed platform (8), where the support (5),

adjustable in length, of the electrospinning capillary tube (3) is supported, to adjust the distance between the electrospinning capillary tube (3) and the electrodes in exposure position (7), with respect to the electrospinning capillary tube (3);

(ii) a central collecting table (17), bounded by the aforementioned peripheral support (15), defining a region of accumulation of electrospun fibres, and also having holes (16), which extend from its surface (19) to a chamber (21);

(iii) a chamber (21), which is inside the central table (17), this chamber being connected by a channel (18) to a vacuum pump (13);

wherein:

- each electrode is capable of being independently exposed (7) or retracted/occluded (14), with respect to the electrospinning capillary tube (3);
- the vacuum pump (13) exerts a controlled vacuum pressure on the holes (16); and
- the central collecting table (17) presents controlled movement, towards the axis of the capillary tube (3), allowing its movement (24) away from or close to the electrospinning capillary tube (3).

The **electrospinning system** (1) comprising the fibre (2) of polymeric material formed by electrospinning from the capillary tube (3) with positive polarity moves by the action of an electric field towards a collector module, which consists of a peripheral support (15) in insulating material where they are longitudinally inserted in holes (6) in its surface (20) multi-electrodes (7) (14) and each electrode (7) (14) equipped with controlled movement towards the axis of the electrospinning capillary tube (3) allowing its exposure (7) or retraction-occultation (14) with respect to the electrospinning capillary tube (3), a central collecting table (17) delimited by the peripheral support (15) of the multi-electrodes which consists of the region of accumulation of the electrospun fibres integrating this holes (16) which extend from its surface (19) to a chamber (21) in its interior being this chamber connected by a channel (18) to a vacuum pump (13) with pressure control, this central collecting table (17) has controlled movement towards the axis of the capillary tube (3) allowing its movement (24) away or close to the electrospinning capillary tube (3).

In one embodiment, this continuous electrospinning system and process comprises the peripheral support (15) of the multi-electrodes mounted on a fixed platform (8) on which the length-adjustable support (5) of the electrospinning capillary tube (3) is supported, allowing the distance between the electrospinning capillary tube (3) and the electrodes in exposure position (7) to the electrospinning capillary tube to be adjusted (3).

In one embodiment, the movement of exposure (22) and retraction-occultation (23) of the electrodes (7) and (14) inserted into holes (6) of the peripheral support (15) to the electrospinning capillary tube (3) results from an electromagnetic force (22) (23) controlled by the computer control unit (9) which develops at the end of the electrode (7) (14) opposite the upper surface (20) of the peripheral support (15) by the action of magnetic flux between a permanent magnet (11) attached to the electrode (7) (14) and an induction coil (10) positioned in the lower region of the peripheral support (15).

In one embodiment, the deposit and alignment of the fibre flow (2) is carried out when one or more pairs of electrodes (7) are held in the exposed position in relation to the electrospinning capillary tube (3), and their respective negative polarities are activated by the computer control unit (8), according to the intended fibre deposition orientation thus forming successive aligned two-dimensional fibre layers (27)(28)(29)(30)(31)(34)(35).

In one embodiment, the retraction-occultation movement of the electrodes (23), with respect to the electrospinning capillary tube (3), towards the interior of its hole (6) in the peripheral support (15) allows the support - deposit of the fibres (27) (28) (29)(30)(31)(33)(35) on the surface (19) of the central collecting table (17) followed by the separation - release of the fibres from the end of the electrodes (7).

The controlled movement (24)(32)(33)(36)(36) away from the surface (19) of the central collecting table (17) of the electrospinning capillary tube (3), after each two-dimensional layer of aligned deposited fibres (27)(28)(29)(30)(31)(33)(35), allows the accumulation of successive fibre layers (27)(28)(29)(30)(30)(31)(33)(35) on the central collecting table (17) thus allowing the formation of a three-dimensional fibre matrix structure (207) in which its thickness (26) is dependent on the number of two-dimensional fibre layers (27)(28)(29)(30)(31)(33)(35) deposited, the fibre thickness and the degree of intended compression between layers by vacuum system action (13).

The successive two-dimensional fibre layers deposited (27)(28)(29)(30)(31)(33)(35) on the central collecting table (17) are maintained in position on the central collecting table (17) by the action of a suction force (25) generated by the vacuum pressure in the surface holes (19) of the central collecting table (17) that communicate with a chamber (21) in its interior connected by a channel (18) to the vacuum pump (13).

The pressure control in the vacuum pump (13) is also intended to control the suction forces (25) on the fibres and the degree of compaction between the formed two-dimensional fibre layers (27)(28)(29)(30)(31)(33)(35) and then the porosity in the perpendicular direction of the deposited fibre layer plane.

Control of the distance between the electrospinning capillary tube (3) with positive polarity through its holder (5) and the exposed multi-electrodes (7) with negative charge, control of the exposure movements (22) and retraction-occultation (23) of the electrodes (7) with negative polarity with respect to the electrospinning capillary tube (3), control of the surface movement (19) of the central collecting table (17) with respect to the electrospinning capillary tube (3), the control of the negative polarity applied to the electrodes (7) and the control of the vacuum pump pressure (13) are carried out by a computer control unit (8) which, depending on the alignment (301) (302) (303) (304) (305) (306) (307) (308)(309) of fibres intended for each two-dimensional layer deposited (27)(28)(29)(30)(31)(33)(35) and the thickness (26) of the matrix, program the sequence of all movements, vacuum pressure and polarity of the required electrodes based on a computational program developed for this purpose.

2. Process of obtaining three-dimensional matrices of aligned polymeric fibres

The process of the present invention is carried out in several steps with the use of the electrospinning system (1), as described in the previous section.

The production of three-dimensional matrices of aligned polymeric fibres (2) occurs when one or more pairs of electrodes (7,14) are exposed to the capillary tube (3), according to the intended fibre orientation, thus forming a two-dimensional layer of aligned fibres, which are kept in adequate position on the central collecting table (17), after the movement of retraction-occultation of the electrodes, by action of the suction force generated by vacuum pressure in their holes.

The controlled movement away from the central collecting table (17) of the electrospinning tube (3), after each deposited fibre layer, allows the accumulation of successive layers and the formation of a three-dimensional matrix with thickness depending on the number of deposited fibre layers, the fibre thickness and the degree of compaction between layers controlled by vacuum pressure.

This way, the production process of aligned three-dimensional matrices of polymeric fibres of the present invention comprises the following steps:

a) exposure of one or more electrode pairs (7) to the capillary tube (3), according to the desired fibre orientation, to form a first layer of aligned fibres (30);

b) application of vacuum pressure to the aligned fibre layer obtained in (a) through the holes (16) of the central collecting table (17), after the retraction-occultation movement of the electrodes (14);
c) separation of the central collecting table (17) from the electrospinning capillary tube (3), by action of movement (33) of the actuator (12);
d) exposure of one or more pairs of electrodes (7) to the capillary tube (3), according to the intended fibre orientation, to form a second layer (31) of aligned fibres;
e) application of vacuum pressure to the layer (31) of aligned fibres, obtained in (d), through the holes (16) of the central collecting table (17), after the movement of retraction-occultation of the electrodes (14).

The production of three-dimensional matrices of aligned polymeric fibres, with more than two layers, is performed by repeating steps (a), (b) and (c), as many times as the number of desired layers, to the process as described above.

Therefore, in order to obtain a matrix with three layers, the process of the present invention comprises the following steps:

a) exposure of one or more electrode pairs (7) to the capillary tube (3), according to the intended fibre orientation, to form a first layer of aligned fibres (30);
b) application of vacuum pressure to the aligned fibre layer obtained in (a) through the holes (16) of the central collecting table (17), after the retraction-occultation movement of the electrodes (14);

c) separation of the central collecting table (17) from the electrospinning capillary tube (3), by action of movement (33) of the actuator (12);
d) exposure of one or more electrode pairs (7) to the capillary tube (3), according to the intended fibre orientation, to form a second layer (31) of aligned fibres;
e) application of vacuum pressure to the layer (32) of aligned fibres, obtained in (d), through the holes (16) of the central collecting table (17), after the movement of retraction-occultation of the electrodes (14);
f) exposure of one or more pairs of electrodes (7) to the capillary tube (3), according to the intended fibre orientation, to form a third layer (33) of aligned fibres;
g) application of vacuum pressure to the layer (33) of aligned fibres, obtained in (f), through the holes (16) of the central collecting table (17), after the movement of retraction-occultation of the electrodes (14).

To obtain matrices with multiple layers and alignment patterns one or more pairs of electrodes are used which are exposed (7) to the capillary tube (3), containing a certain polymeric solution, where the composition and concentration of the polymer in solution, as well as the solvents used, vary according to the purpose for which the matrix is intended.

To the electrodes, located in superficial holes (16) and radially distributed around the central collecting table (17), with a certain area, a certain negative voltage is applied, also selected according to the type of fibre and matrix to produce.

Each one of the different alignments of the two-dimensional fibre layers is obtained by the sequential exposure (7), of a number of selected electrodes, to the electrospinning capillary tube (3) during a certain period, also selected according to the purpose for which the fibres and the matrices are intended.

The different layers of deposited two-dimensional fibres thus result from the performance of consecutive cycles of various types of alignment, which is obtained by combining the variables or production factors described above.

It is thus possible to obtain several fibre alignments (301) (302) (303) (304) (305) (306) (307) (308) (309) sequentially deposited in layers, each one of the alignments being obtained by exposure (7) of different electrodes to the spinning capillary tube (3).

The set of different layers, differently aligned, forms an alignment pattern (200), which is repeated successively between the first (203), intermediate (205) and last (206) pattern, thus resulting in the thickness (26) of the three-dimensional matrix, which is delimited by the dimensions (202) and (201) of the central collecting table (17).

The production process of three-dimensional matrices of aligned polymeric fibres is carried out continuously by successively carrying out the various steps for the formation of two-dimensional layers of polymeric fibres, according to the above described.

3. Characterization of the three-dimensional matrices of aligned polymeric fibres

The thickness of the obtained matrices varies therefore, not only in function of the type and quantity of polymer used, but also in function of the number of deposited fibre layers, the thickness of these fibres and the degree of compaction between layers, each one of these aspects being controlled by vacuum pressure exerted on the fibre layers deposited in the central collecting table.

On the other hand, the alignment of the fibres in each layer is controlled by the different movements and number of electrodes exposed and/or concealed in each cycle of fibre formation.

In conclusion, through the implementation of the system and the process of the present invention, it is possible to obtain three-dimensional matrices of aligned polymeric fibres, which can present several alignment patterns, along the thickness of the matrix, which can also be variable.

4. Matrix applications

Example: Production of a polymeric matrix of aligned fibres

This example refers to the production of a matrix composed of 27 layers of aligned polymeric fibres for cartilage engineering, with a total thickness of 3,24 mm.

The polymer used to manufacture the matrix was polycaprolactone (PCL) with a molecular weight of 80,000 Da.

PCL was dissolved at concentrations of 12% dichloromethane (DCM) and dimethylformamide (DMF) at a ratio of 1:1 (v:v) after 12 hours of stirring at room temperature.

Then, the molten polymer was electrospun using a capillary tube (3) with a flow of 2,5 mL/h, a voltage of 25 kV and a working distance of 15 cm to the central collecting table (17).

In this configuration of the electrospinning system (1), the central collecting table (17) has a diameter of 8mm, and the holes (16) on its surface are subjected to a vacuum pressure of 3300 Pa.

Eleven electrodes (701), (702), (703), (704), (705), (706), (707), (708), (709), (710) and (711) distributed radially around the table (as shown in figure 8) were used for the formation of each layer of fibres, each electrode having an average diameter of 1,5 mm.

In their exposure position (7) to the electrospinning capillary tube (3) the said electrodes were subjected to a negative voltage of -3kV.

Each of the different alignments of the two-dimensional fibre layers was obtained by sequential exposure (7) of pairs of electrodes to the electrospinning capillary tube (3) for 2.3 min.

The 27 layers of deposited two-dimensional fibres resulted from the performance of 3 consecutive cycles (3 times) of 9 alignment patterns (301), (302), (303), (304), (305), (306), (307), (308) and (309) in this order, as follows:

- the alignment (301) obtained by exposure (7) of the electrodes (702) and (710);
- the alignment (302) obtained by exposure (7) of the electrodes (702) and (705);
- the alignment (303) obtained by exposure (7) of the electrodes (701) and (706);
- the alignment (304) obtained by exposure (7) of the electrodes (701) and (706);
- the alignment (305) obtained by exposure (7) of the electrodes (708) and (702);
- the alignment (306) obtained by exposure (7) of the electrodes (706) and (711);
- the alignment (307) obtained by exposure (7) of the electrodes (710) and (707);
- the alignment (308) obtained by exposure (7) of the electrodes (709) and (702); and
- the alignment (309) obtained by exposure (7) of the electrodes (710) and (703).

At the end of the deposition of each layer of aligned fibres, the central collecting table (17) has moved away from the electrospinning capillary tube about 0,12 mm, this being the value corresponding to the average thickness of each layer of deposited dimensional fibres.

In total, the central collecting table moved away about 3,24 mm, corresponding to the thickness of the matrix obtained at the end of the 27 deposited layers.

The three-dimensional matrix of aligned fibres obtained in this example shows, like the native cartilage, a preferential alignment of the fibres in its surface area parallel to the surface, in the intermediate area there is no preferential alignment, and in the deepest area the fibres are aligned in a vertical manner to the surface.

CLAIMS

1. An **electrospinning system** (1) comprising (a) a fibre-forming module, (b) a multi-electrode array, (c) a collector module, for collecting deposited fibres, a power supply, a vacuum pump (13), **characterised in that:**

a) The fibre-forming module consists of an electrospinning capillary tube (3) with positive polarity;

b) The **multi-electrode array** is inserted in a peripheral support (15) and comprises:

   - several electrodes (7) (14), each one being equipped with controlled movement towards the axis of the electrospinning capillary tube (3);
   - several magnets (11), each of which is attached to an electrode (7,14) for generating electromagnetic force (22, 23), together with the induction coil (10), controlled by the computer unit (9);

c) The **collector** module comprises:

   - a peripheral support (15), where the electrodes (7) (14) are inserted longitudinally into holes (6) on its surface (20), the said support (15) being mounted on a fixed platform (8), where the length adjustable support (5) of the electrospinning capillary tube (3) is supported, to adjust the distance between the electrospinning capillary tube (3) and the electrodes in exposure position (7), with regard to the electrospinning capillary tube (3);
   - a central collecting table (17), bounded by the aforementioned peripheral support (15), defining a region of accumulation of electrospun fibres, and also having holes (16), which extend from its surface (19) to a chamber (21);

- a chamber (21), which is located inside the central table (17), this chamber being connected by a channel (18) to a vacuum pump (13);

wherein:

- each electrode is capable of being independently exposed (7) or retracted/occluded (14), with respect to the electrospinning capillary tube (3);
- the vacuum pump (13) exerts a controlled vacuum pressure on the holes (16); and
- the central collecting table (17) presents controlled movement, towards the axis of the capillary tube (3), allowing its movement (24) away from or close to the electrospinning capillary tube (3).

2. **Electrospinning system** (1) according to the previous claim comprising computer control unit (9) and computer program.

3. A **process for the production** of three-dimensional matrices of continuously aligned polymeric fibres that occurs in the system of any of claims 1 or 2 **characterized by** comprising the following steps:

a) exposure of one or more pairs of electrodes (7) to the capillary tube (3) containing a solution of a certain polymer suitable for the function of the matrix to be produced, this exposure being made according to the intended fibre orientation, by applying a negative voltage to the selected electrodes, during a certain time, to form layers of aligned fibres (30);

b) application of vacuum pressure to the aligned fibre layer obtained in (a) through the holes (16) of the central collecting table (17), with the movement of retraction-occultation of the electrodes (14);

c) separation of the central collecting table (17) from the electrospinning capillary tube (3), by action of movement (33) of the actuator (12);

d) repetition of electrode pair sequential exposure cycles (7) to the electrospinning capillary tube (3) from (a), application of vacuum pressure to the aligned fibre layer (b) and separation of the central collecting table (17) from the electrospinning capillary tube (3) from (c), as many times as necessary to form a two-dimensional fibre layer matrix (2), with the required alignment;

e) repetition of the cycles, as described in steps (a), (b) and (c), as many times as necessary, in which at least one of the electrospinning factors is modified from those defined in step (a), (b) or (c) to form a matrix of two-dimensional fibre layers (2), with the required alignment, this alignment being different from that obtained in steps (a), (b), (c) and (d);

f) repetition of exposure cycles from (e) as many times as necessary, in which at least one of the electrospinning factors is modified from those defined in the previous cycle to form a matrix of two-dimensional fibre layers (2), with the required alignment, this alignment being different from that obtained in the previous cycle

wherein:

the controlled movement away from the central collecting table of the electrospinning tube after each layer of deposited fibres allows the accumulation of successive layers and the formation of a three-dimensional matrix with thickness depending on the number of fibre layers deposited, the thickness of the fibres and the degree of compaction between layers is controlled by vacuum pressure and the vacuum pressure secures the fibres to the table.

4. **Process for the production** of three-dimensional matrices of continuously aligned polymeric fibres according to the previous claim, which occurs in the system of any of the claims 1 or 2 characterised by the peripheral support having a multi-electrode array and each electrode has individual movement controlled towards the axis of the electrospinning capillary tube, comprising two positions, one of exposure and another of retraction-occultation with respect to the electrospinning capillary tube.

5. **Process for the production** of three-dimensional matrices of continuously aligned polymeric fibres according to any of the previous claims 3 or 4, which occurs in the system of any of the claims 1 or 2 **characterized by** the central collecting table being delimited by the peripheral support of the multi-electrodes consisting of the region of accumulation of electrospun fibres integrating these holes that extend from its surface to a cavity inside it being this cavity connected to a vacuum pump with pressure control, in which the central collecting table has controlled movement towards the axis of the capillary tube allowing its separation or approach to the electrospinning capillary tube.

6. **Process for the production** of three-dimensional matrices of continuously aligned polymeric fibres according to any of the previous claims 3 to 5, which results in the system from any of the claims 1 or 2 **characterised by** the exposure and retraction-occultation movement of the electrodes inserted into holes in the peripheral support to the electrospinning capillary tube resulting from a controlled electromagnetic force which develops at the

end of the electrode opposite the upper surface of the peripheral support by the action of magnetic flux between a permanent magnet attached to the electrode and an induction coil positioned in the lower region of the peripheral support.

7. **Process for the production** of three-dimensional matrices of continuously aligned polymeric fibres according to any of the previous claims 3 to 6, which occurs in the system of any of the claims 1 or 2 **characterized by** the deposit and alignment of the flow of fibres being performed when one or more pairs of electrodes are held in the exposed position with regard to the electrospinning capillary tube, and their respective negative polarities are activated, according to the intended orientation of the fibre deposition thus forming a layer of aligned two-dimensional fibres.

8. **Process for the production** of three-dimensional matrices of continuously aligned polymeric fibres according to any of the previous claims 3 to 7, which occurs in the system of any of the claims 1 or 2 **characterized by** the movement of retraction-occultation of the electrodes, with regard to the electrospinning capillary tube, to the inside of its hole in the peripheral support to support and deposit the fibres on the central collecting table followed by the separation of the fibres from the ends of the electrodes.

9. **Process for the production** of three-dimensional matrices of continuously aligned polymeric fibres according to any of the previous claims 3 to 8, which occurs in the system of any of the claims 1 or 2 **characterized by** the

controlled movement away from the surface of the central collecting table of the electrospinning capillary tube, after each two-dimensional layer of aligned fibres, accumulate successive layers of fibres on the central collecting table for the formation of a three-dimensional fibre matrix structure in which its thickness is dependent on the number of two-dimensional fibre layers deposited, the fibre thickness and the degree of compaction between layers required by the system action and vacuum pressure.

10. **Process for the production** of three-dimensional matrices of continuously aligned polymeric fibres according to any of the previous claims 3 to 9, which occurs in the system of any of the claims 1 or 2 **characterized by** the successive layers of deposited fibres in the collector module being kept in position in the central collecting table, by action of the suction force generated in the holes of the surface of the central collecting table by action of vacuum pressure generated in the inner chamber of the central collecting table that communicate with the vacuum pump.

11. **Process for the production** of three-dimensional matrices of continuously aligned polymeric fibres according to any of the previous claims 3 to 10, which occurs in the system of any of the claims 1 or 2 **characterized by** the pressure control in the vacuum pump controlling the degree of compaction between the formed two-dimensional fibre layers and the porosity in the perpendicular direction of the deposited fibre layer plane.

12. **Process for the production** of three-dimensional arrays of continuously aligned polymeric fibres according to any of the previous claims 3 to 11, which occurs in the system of any of the claims 1 or 2 **characterised by** the parameters being controlled by a computer control unit (9) and the movements of the electrodes being controlled by a computer program.

13. Three-dimensional **matrices** of aligned polymeric fibres **characterized by** 27 layers of two-dimensional polymeric fibres, with 9 alignment patterns (301), (302), (303), (304), (305), (306), (307), (308) and (309), with a total thickness of 3.24 mm.

14. Three-dimensional **matrices** of aligned polymeric fibres, according to the previous claim, **characterised by** a preferential alignment of the fibres, (i) in parallel in their surface area, (ii) without any preferential alignment in the intermediate area, and (iii) the fibres are aligned in a vertical manner to the surface in the deepest area.

15. Three-dimensional **matrices** of aligned polymeric **fibres, as described in any of the claims 13 or 14, characterized by** being applied in medicine, in regenerative medicine and/or in cartilage engineering.

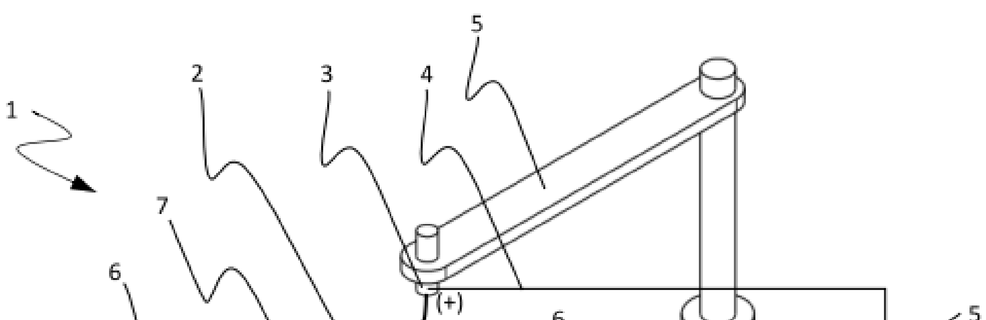

Fig. 1

Fig. 1

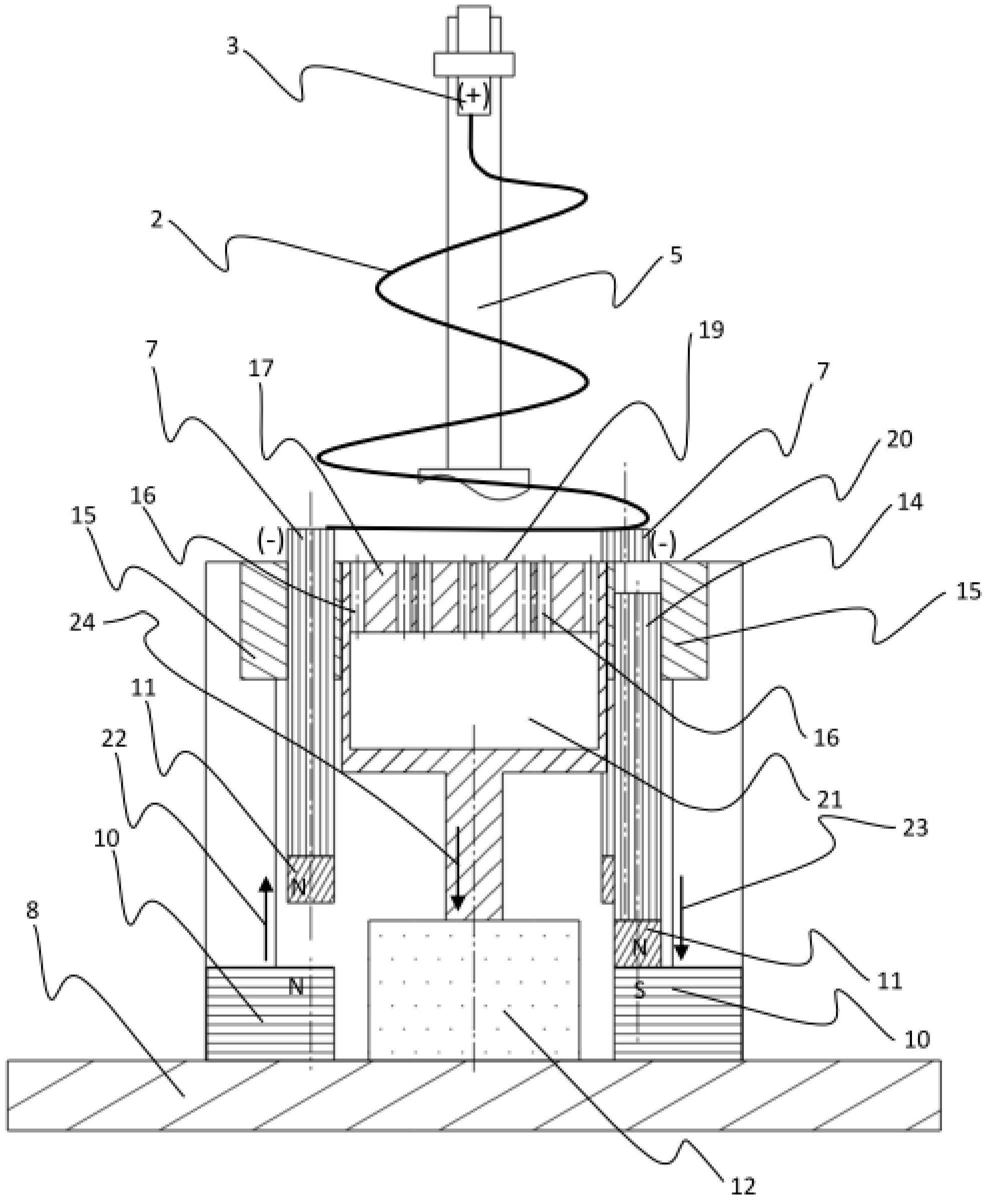

Fig. 2

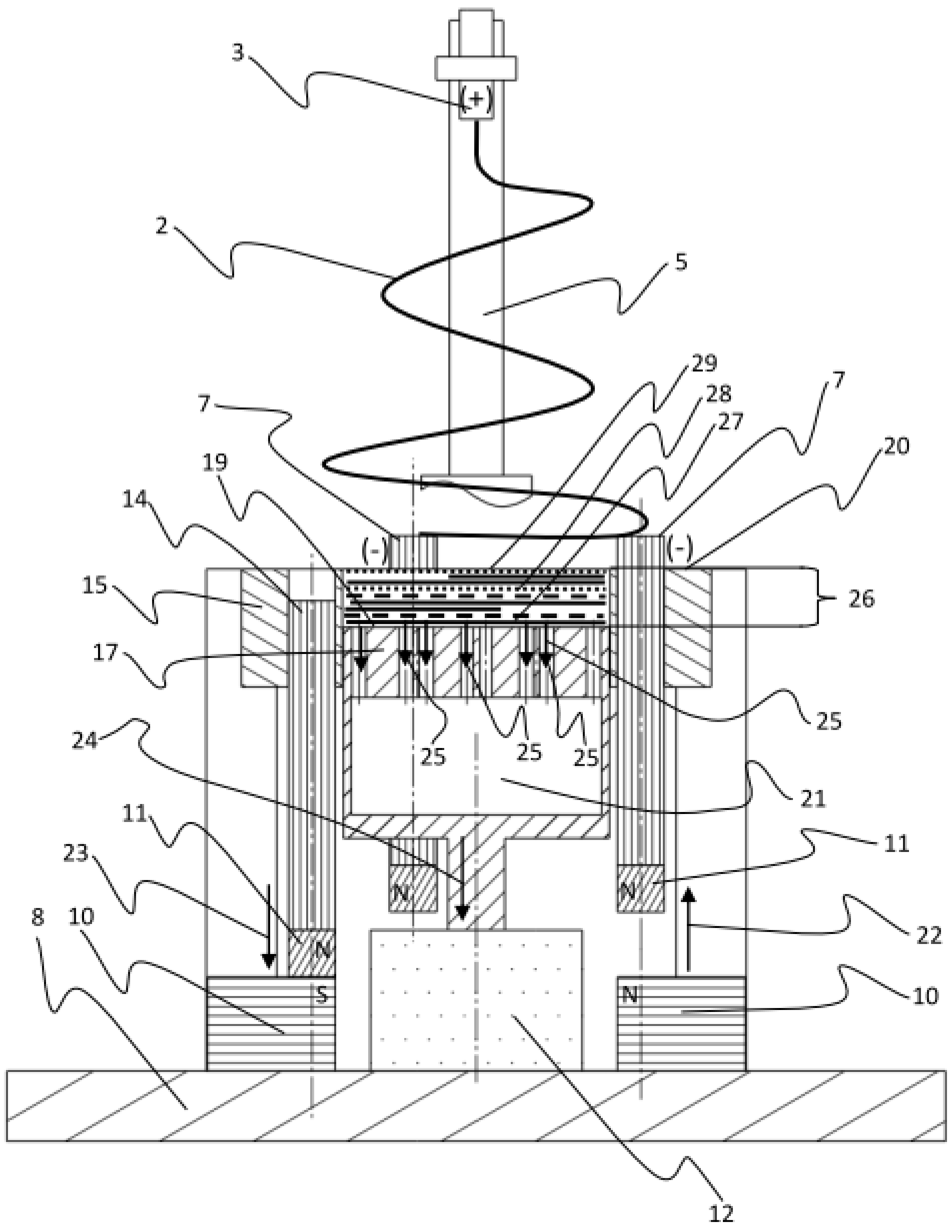
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Fig. 3

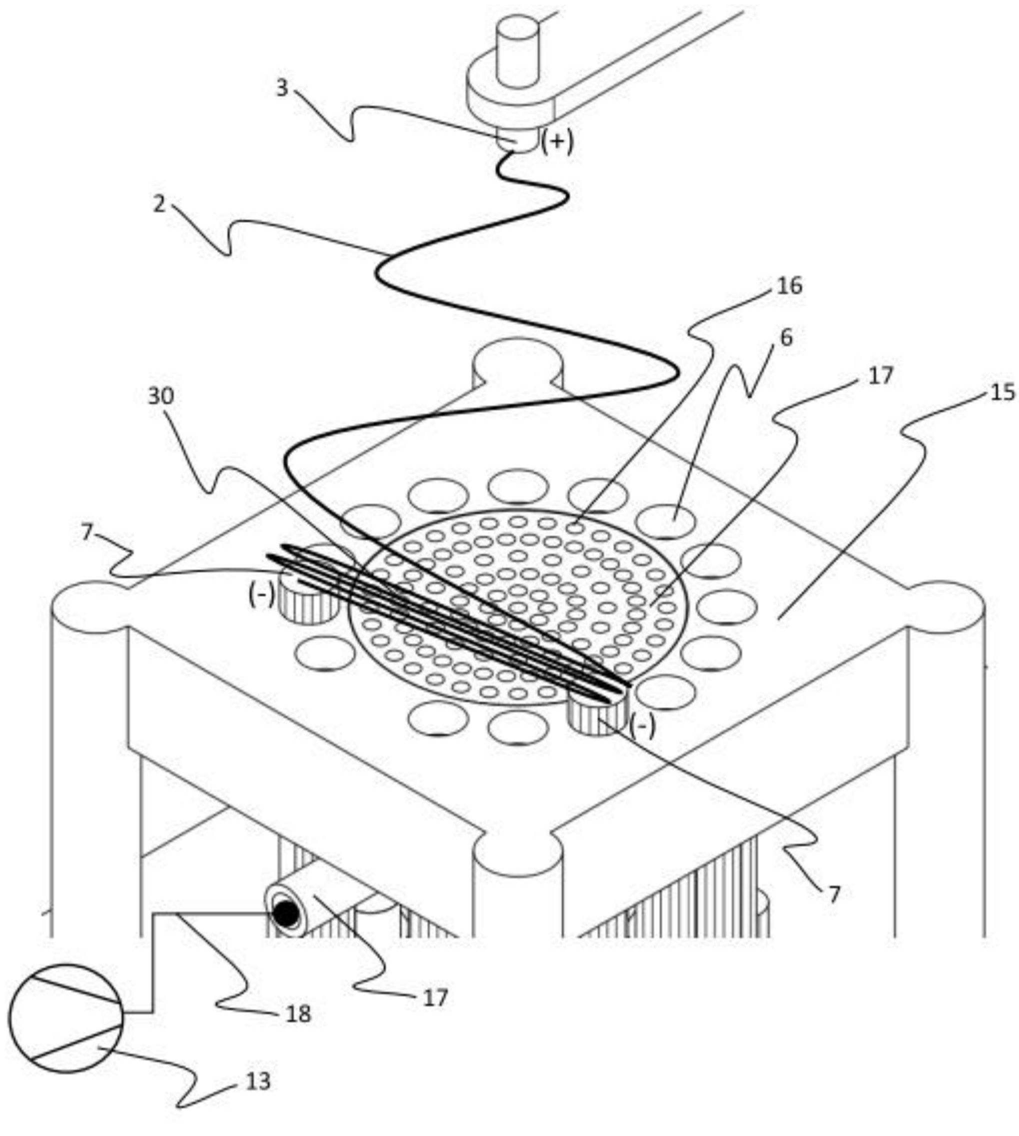

Fig. 4

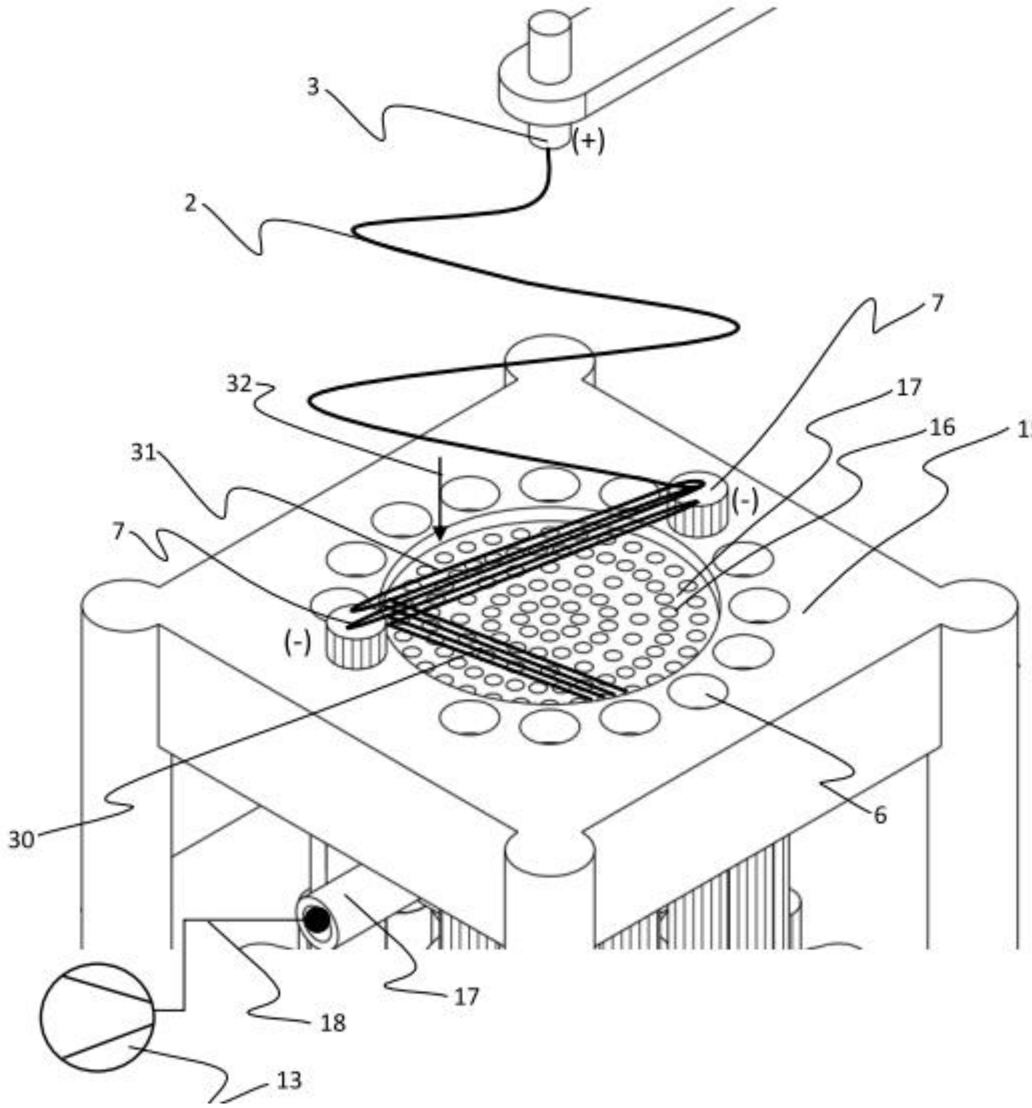

Fig. 5

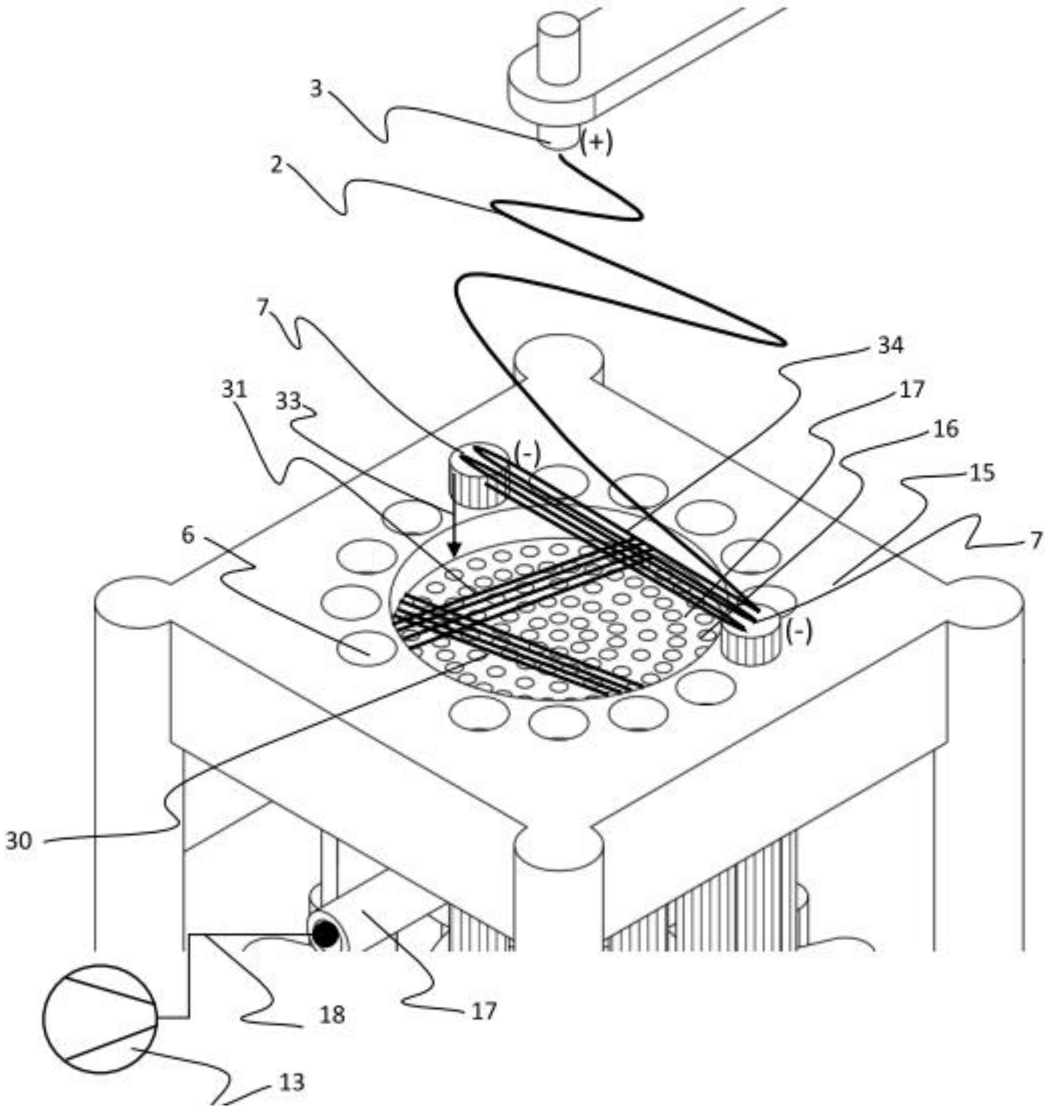

Fig. 6

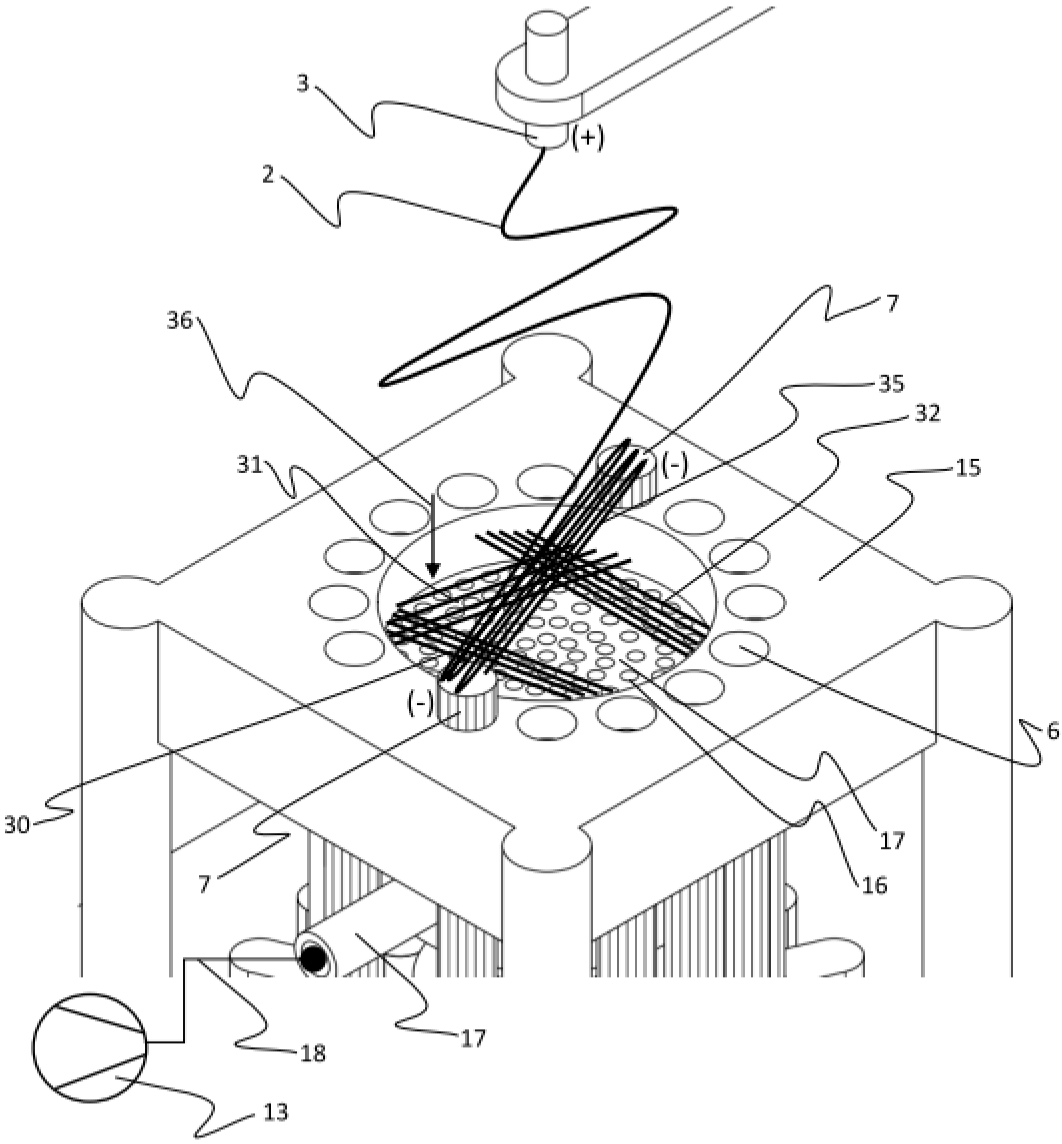

Fig. 7

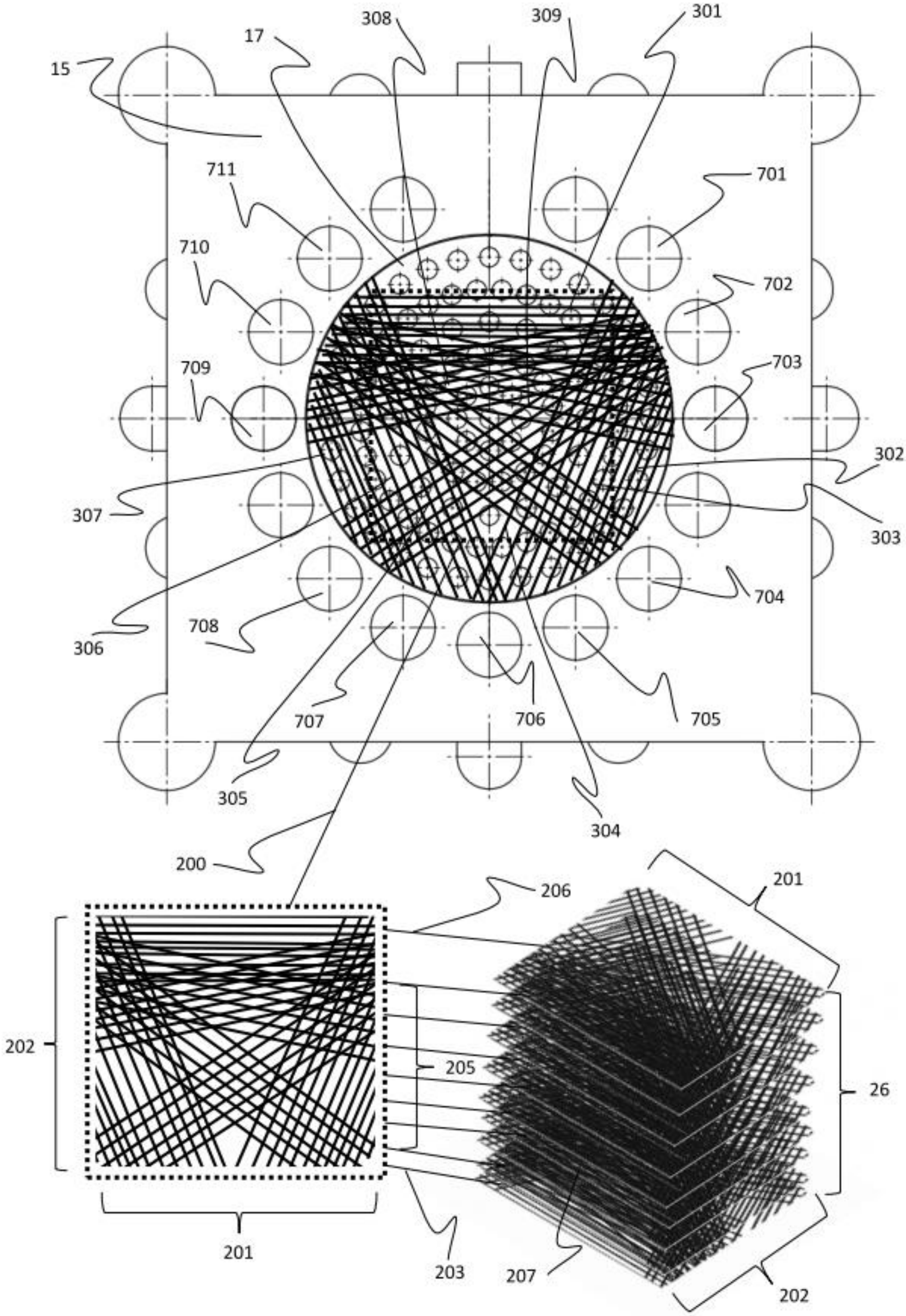

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Fig. 8