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Title:

AUTOMATED MANUFACTURING OF THREE-DIMENSIONAL CELL MATRICES WITH NANOFIBRES OF CONTROLLED ALIGNMENT AND UNIFORM CELL DISTRIBUTION

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Technical field of the invention

[0001] This invention relates to a system and a process for the automated manufacture of three-dimensional cell matrices by electrospinning from nanofibres of controlled alignment and uniform cell distribution throughout their thickness.

[0002] From the implementation of the invention it is possible to obtain, in a totally automatic way and without manual intervention, three-dimensional cell matrices of aligned polymeric fibres, with a uniform cell distribution, throughout the thickness of the matrix, which may present several patterns of alignment of the nanofibres, throughout the thickness, being the cells of the selected tissues seeded, in an alternated way, over the layers of two-dimensional meshes of nanofibres, deposited throughout the thickness, thus making the matrix thickness to be dependent of the number of layers of deposited fibres, of the thickness of the fibres and of the degree of compaction between layers.

[0003] In this way, the present invention has many applications in various areas, in the manufacture of products or structures, on a nanometric scale, which depend on high surface area, such as in biotechnology, in the pharmaceutical and in tissue engineering areas, in particular in regenerative medicine.

Prior art

[0004] 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.

[0005] 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.

[0006] 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.

[0007] Currently, the key configuration of a generic electrospinning 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.

[0008] 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.

[0009] 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.

[0010] 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.

[0011] Oriented fibre networks have the possibility of developing anisotropic properties in materials. These relationships are quite obvious in the field of tissue engineering.

[0012] 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, in order to allow the development of the three-dimensional cell matrix throughout the depth of the matrix.

[0013] 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 .

[0014] 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.

[0015] 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 throughout deposited nanofibres, which favoured the parallel arrangement between them, due to electrostatic repulsion.

[0016] 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).

[0017] 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.

[0018] 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 .

[0019] 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.

[0020] 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.

[0021] 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.

[0022] In addition to the problem described, related to the formation of thick three-dimensional matrices with controlled alignment of the nanofibres throughout their thickness, other problems occur when seeding this type of nanofibre matrices with cells for tissue engineering purposes, as the seeding process is usually a manual process in which the cells are manually deposited on the surfaces of the previously manufactured three-dimensional nanofibre matrices).

[0023] The manual seeding operation of the three-dimensional nanofibre matrices requires intensive laboratory labour with high costs and contamination risks, being a manual process, it presents a very low reproducibility in terms of the uniformity of the deposited cells distribution. All these limitations associated to the seeding process contribute decisively to limit the large-scale production of these cell matrices.

[0024] Another limitation associated with the manual seeding process of three-dimensional matrices of nanofibres, with thicknesses in the order of several millimetres, is that it is not possible to control the distribution of cells throughout the thickness/depth of the three-dimensional matrix, as these

are essentially accumulated in the surface fibres of the three-dimensional matrix, thus leaving the core of the three-dimensional matrix without or with few cells.

[0025] This effect leads to the extracellular matter, produced by the cells during the culture period, being essentially concentrated in the peripheral regions of the three-dimensional nanofibre matrix, leaving the core without extracellular matter and thus creating tissues with highly anisotropic mechanical properties that limit or even prevent their application in the clinical and/or medical field.

[0026] For specific applications, such as tissue engineering, it is necessary to form cell matrices of nanofibres, with a high thickness, i.e. in the order of several millimetres, with control of the alignment of the fibres throughout the thickness, with a uniform distribution of cells throughout the volume of the three-dimensional nanofibre matrix, with control of the distance between aligned nanofibres (inplane porosity) in the deposition plane forming two-dimensional nanofibre meshes and control of the degree of compaction (porosity throughout the thickness) between layers of the deposited two-dimensional nanofibre meshes.

[0027] The porosity of these nanofibre matrices is of utmost relevance in enhancing cell migration and multiplication, as well as the delivery of nutrients to the cells, throughout the volume of the three-dimensional nanofibre matrix.

[0028] Thus, there is a need to develop a system and to implement an automated manufacturing process of three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the

thickness, which allows the production of various alignment patterns of the fibres throughout the matrix thickness and enables a uniform distribution of the cells throughout the matrix thickness.

[0029] The present invention proposes to solve the problems of the prior art, described above, through the implementation of a system and a process for the automated manufacture of three-dimensional cell matrices, which can present various patterns of fibre alignment, throughout the thickness of the matrix and with a uniform distribution of cells throughout the thickness of the matrix, this thickness being dependent on the number of layers of fibres deposited, the thickness of the fibres and the degree of compaction between layers.

Summary of the invention

[0030] The present invention relates to a system and process for the automated manufacture of three-dimensional cell matrices, by electrospinning, from nanofibres with controlled alignment and uniform cell distribution throughout their thickness.

[0031] The system of the present invention comprises a module (A) for forming nanofibres by electrospinning, a module (B) for collecting the formed nanofibres, a module (C) for deposition of the collected nanofibres, a module (D) for electropulverisation of cells, a vacuum pump and a computational unit according to claim 1.

[0032] The configuration of this system, in particular the fact that the module (B), nanofibre collector, comprises two collecting cylinders, with coaxial axes and perpendicular to the axis of the electrospinning capillary tube, where each

cylinder is provided with continuous rotation movement by an electric motor controlled by a computerised unit, in which the electrospun nanofibres are collected on the surfaces and between the surfaces of the collecting cylinders, also comprising this module (B), brushes for removing the nanofibres, which remain on the cylindrical surfaces, and where the module (C), of fibre deposition, comprises a deposition table, able to be moved linearly, in the direction parallel to its surface and in the direction of the axis of the electrospinning capillary tube, and rotationally, around its longitudinal axis, assures that the electrospinning process occurs in a continuous and automatic way, with formation of three-dimensional matrices with uniform distribution of the cells throughout the thickness of the three-dimensional cell matrix and with alignment and distance between nanofibres controlled according to the desired result.

[0033] Furthermore, the presence of module (D), for electropulverisation of cells, makes it possible for the tissue engineering process, by electrospinning, to take place in a continuous and automated manner, capable of producing three-dimensional cell matrices suitable for use in medicine, regenerative medicine, cartilage engineering, etc., according to claim 15.

[0034] In another aspect, the present invention relates to a continuous and automated process for forming three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout their thickness, according to claim 3.

[0035] The process of the present invention allows to obtain, in a totally automatic way and without manual intervention,

three-dimensional cell matrices of aligned polymeric fibres, with a uniform cell distribution throughout the thickness of the matrix, which can present several alignment patterns, of the nanofibres that compose them, throughout their thickness, being the thickness of the matrix dependent on the number of fibre layers deposited, the thickness of the fibres and the degree of compaction between layers.

[0036] This process has the additional advantage of being versatile, simple, and operating in automatic and continuous mode, so it is not necessary to produce a series of layers of nanofibre meshes with a certain alignment, to manually add other layers with different alignments and to avoid the laborious manual process of cell seeding of the three-dimensional matrices of nanofibres previously manufactured, overcoming the limited capacity of this manual process to ensure the uniformity/control of the cells distribution in the three-dimensional matrices of nanofibres throughout the thickness.

[0037] This limitation, which leads to the extracellular matter produced by the cells, during the culture period, being essentially concentrated in the peripheral regions of the three-dimensional matrix of nanofibres, leaving the nucleus of the matrix without extracellular matter and thus creating tissues with highly anisotropic mechanical properties, thus limiting or even preventing its clinical adoption, is thus completely overcome.

Brief description of the drawings

[0038]

Figure 1 : Schematic depiction of an embodiment of a manufacturing system according to the present invention, wherein the following figures represent:

6

collector cylinder of the collector module;

10

deposition table positioned on the collector module;

13

electropulverisation capillary tube;

22

direction and orientations of the linear movement of the deposition table parallel to its surface;

24

orientation of the rotary movement of the deposition table around its longitudinal axis;

30

collector cylinder of the collector module;

Figure 2 : Schematic depiction of an embodiment of a manufacturing system according to the present invention, wherein the following figures represent:

1

manufacturing system;

2

container/syringe with the polymer to be electrospun;

3

electrospinning capillary tube support;

4

electrospinning capillary tube of the electrospinning module;

5

electrospun nanofibre on the cylindrical surfaces (33, 35) of the collector cylinders (6, 29);

6

collector cylinder of the collector module;

7
motor of the collector cylinder (6);
8
nanofibres deposited on the deposition table (10);
9
surface of the deposition table (10) with holes, which connect to the chamber in its interior (not depicted);
10
deposition table positioned on the collector module;
11
connecting channel between the inner chamber (not shown) of the deposition table (10) and the vacuum pump (21) ;
12
collector ring of the electropulverisation module;
13
electropulverisation capillary tube;
14
container/syringe with cells in suspension to be electropulverised;
15
support for the electropulverisation capillary tube;
16
connection of positive polarity between the electropulverisation capillary tube (13) and the power supply (17);
17
power supply of the electropulverisation module;
18
connection of negative/neutral polarity between the collector ring (12) and the power supply (17);
19
deposition table positioned on the electropulverisation module;

20
computerised control unit;
21
vacuum pump;
22
direction and orientations of the linear movement of the deposition table parallel to its surface;
23
carriage of the linear movement of the deposition table parallel to its surface;
24
orientation of the rotation movement of the deposition table around its longitudinal axis;
25
plate of the rotation movement of the deposition table around its longitudinal axis;
26
carriage of the deposition table movement towards the electrospinning capillary tube;
27
direction and orientations of the deposition table movement towards the electrospinning capillary tube;
28
support for the collector cylinders (6, 30);
29
motor of the collector cylinder (30);
30
collector cylinder of the collector module;
31
rotation orientation of the collector cylinders;
32
collector cylinder cleaning brush (30);
33

negative/neutral polarity connection between the collector cylinders (6, 30) and the power supply of the collector module (17);

34

conductive cylindrical surface of the collector cylinder (30);

35

collector cylinder cleaning brush (6);

36

conductive cylindrical surface of the collector cylinder (6);

37

power supply for the electrospinning module;

38

positive polarity connection between the electrospinning capillary tube and the power supply of the electrospinning module (17);

39

carriage motor of the linear movement of the deposition table parallel to its surface;

40

plate motor of the rotation movement of the deposition table around its longitudinal axis;

41

carriage motor of the deposition table movement towards the electrospinning capillary tube;

Figure 3 : Schematic depiction of a part of the manufacturing system (1), with partial sectional representation of the deposition table (10) according to a preferred embodiment of the present invention, wherein:

the electrospun nanofibre (5) is collected on and between the cylindrical surfaces (34, 36) of the collector cylinders (6, 30) in a continuous manner by action of the rotation movement (31) of the collector cylinders (6, 30), being the portion of the nanofibres formed between the cylindrical surfaces (34,

36) continuously deposited (8) on the surface with holes (9) of the deposition table (10) with the holes being connected to the chamber (42) in its interior, which is subjected to vacuum pressure through the connection channel (11) to the vacuum pump (not shown);
the orientation of the suction force (43), of attachment-compaction of the nanofibres (8), generated by the effect of the vacuum pressure present in the chamber (42);
the nanofibres (8) attached to the deposition table (10) by action of the suction force (43) generated by the vacuum on the surface with holes (9) of the deposition table (10) are disrupted by the stretching effect of the part attached to the surfaces (34, 36) of the collector cylinders (6, 30) by action of the continuous rotation movement (31) thereof.
the nanofibres (8) deposited on the deposition table (10) form a mesh with a distance between the nanofibres (44) controlled by the linear movement (22) of the deposition table parallel to its surface;
Figure 4 : Schematic depiction of one of the embodiments of the electrospinning process of the invention, in which:
the electrospun nanofibre (5) collected by the cylindrical surfaces (34, 36) of the collector module is continuously deposited on the surface with holes (9) of the deposition table (10) forming a first two-dimensional mesh of aligned nanofibres (81) being the distance between the deposited nanofibres (45) and the size of the formed mesh controlled by the linear movement (46) of the deposition table (10) parallel to its surface (9), the mesh (81) being attached to the deposition table by action of the vacuum suction force (43) generated on the surface with holes (9) of the deposition table (10).
Figure 5 : Schematic depiction of the two-dimensional nanofibre mesh of the first layer (81) which is followed by the deposition of the second two-dimensional mesh layer of aligned

nanofibres (82), the alignment of this mesh (82) being controlled by the rotation movement (49) of the deposition table (10) and the distance between the deposited nanofibres (47) and the size of the formed mesh being controlled by the linear movement (48) of the deposition table (10) parallel to its surface (9), the two meshes (81, 82) being attached to the deposition table by action of the vacuum suction force (43) generated on the surface with holes (9) of the deposition table (10).

Figure 6 : Schematic depiction of the two-dimensional nanofibre mesh of the first layer (81) and second layer of two-dimensional nanofibre mesh (82) which is followed by the deposition of the third two-dimensional mesh layer of aligned nanofibres (83), the alignment of this mesh (83) being controlled by the rotation movement (51) of the deposition table (10) and the distance between the deposited nanofibres (49) and the size of the formed mesh controlled by the linear movement (50) of the deposition table (10) parallel to its surface (9), the three meshes (81, 82, 83) being attached to the deposition table by action of the vacuum suction force (43) generated on the surface with holes (9) of the deposition table (10).

Figure 7 : Schematic depiction of the two-dimensional nanofibre mesh of the first layer (81), second layer (82) and third layer (83) which is followed by the deposition of the fourth two-dimensional mesh layer of aligned nanofibres (84), the alignment of this mesh (84) being controlled by the rotation movement (54) of the deposition table (10) and the distance between the deposited nanofibres (52) and the size of the formed mesh controlled by the linear movement (53) of the deposition table (10) parallel to its surface (9), the four meshes (81, 82, 83 and 84) being attached to the deposition

table by action of the vacuum suction force (43) generated on the surface with holes (9) of the deposition table (10).

Figure 8 : Schematic depiction of the manufacturing system (1) according to a preferred embodiment of the present invention, wherein:

the linear movement (55) of the deposition table (10) from the position on the nanofibre collector module, after the deposition of the four layers of two-dimensional nanofibre meshes (81, 82, 83 and 84) to the position on the electropulverisation module (19) being positioned concentrically with the collector ring (12);

the four layers of nanofibre meshes (81, 82, 83 and 84) on the deposition table are seeded with cells through the electropulverisation cone (56) that forms between the electropulverisation capillary tube (13) with positive polarity (16) and the collector ring (12) with negative/neutral polarity (18);

during the time the cells are electropulverised over the four mesh layers (81, 82, 83 and 84) the deposition table in the electropulverisation position (19) will rotate continuously (57) to uniformly distribute the cells on the deposited meshes;

during the electropulverisation period of the cells the vacuum pressure in the holes of the surface (9) of the deposition table (19) is switched off.

Figure 9 : Schematic depiction of the manufacturing system (1) according to a preferred embodiment of the present invention, wherein:

the linear movement (59) of the deposition table from the position on the electropulverisation module (19) to the position on the nanofibre collector module (10) after electropulverisation of the cells (58) over the four layers of two-dimensional nanofibre meshes (81, 82, 83, 84) ;

activation of the suction force (43) generated by the vacuum in the holes of the surface (9) of the deposition table (10), allowing the attachment of the deposited meshes (81,82,83,84) and of the future meshes to be deposited (85,86,87,88);
linear movement (60) of the deposition table (10) in the direction and opposite orientation to the electrospinning capillary tube (4) allowing the deposition of new two-dimensional nanofibre mesh layers (85,86,87,88) over the mesh layers (81,82,83 and 84) and electropulverised cells (58) preceding them;
new formation of the electrospun nanofibre (5) on and between the cylindrical surfaces (34, 36) of the collector cylinders (6, 30) in continuous rotation movement (31), allowing continuous deposition of new two-dimensional nanofibre mesh layers (85,86,87,88) which will be alternately electropulverised with cells (58).
Figure 10 : Schematic depiction of the top view of the deposition table (10) with the representation of eight two-dimensional nanofibre meshes with different alignments (81) (82) (83) (84) (85) (86) (87) (88), sequentially deposited in layers, and after the sequential deposition of the meshes (81) (82) (83) (84) these meshes are electropulverised with cells (58) and then the two-dimensional nanofibres meshes (83) (84) (85) (86) were deposited and afterwards the cells (58) are electropulverised over them, whereby the set of these eight mesh layers with cells form a pattern of alignments (63), which is repeated successively, between the first (66), intermediate (65) and last (64) pattern, thus resulting in the thickness (67) of the nanofibre three-dimensional cell matrix (68), which is delimited by the dimensions (61) and (62) .
General description of the invention
[0039] This invention relates to a system and a process for the automated manufacture of three-dimensional cell matrices,

obtaining nanofibres with controlled alignment and uniform cell distribution throughout their thickness, with the possibility of producing various fibre alignment patterns throughout the matrix thickness with controlled thickness and uniform cell distribution throughout the matrix thickness, this thickness being dependent on the number of layers of deposited nanofibre meshes, the thickness of the nanofibres and the degree of compaction between layers.

[0040] The system of the present invention comprises a module (A) for forming nanofibres by electrospinning, a module (B) for collecting the produced nanofibres, a module (C) for deposition of the collected nanofibres, and a module (D) for electropulverisation of cells.

[0041] Module (A) comprises a syringe to contain a polymer solution, connected to an injector pump, connected to an electrospinning capillary tube, which is connected to a voltage source, configured to provide positive polarity. Module (A) is aligned perpendicularly with the axes of the two collector cylinders of module (B), the nanofibre collector.

[0042] Module (B) comprises two collector cylinders, each equipped with a continuous rotation movement, through the action of an electric motor, which is controlled by a computerised unit. The distance between the top faces of the two cylinders is equal to the diameter of the nanofibre deposition table of module (C), and the cylindrical surfaces of the collector cylinders are made of conductive material, and may have negative or neutral polarity.

[0043] The nanofibre of polymeric material, formed by electrospinning from the capillary tube of the module (A),

with positive polarity, moves by the action of an electric field towards a collector module (C), and these are collected on the cylindrical surfaces and between the cylindrical surfaces of the collector cylinders, which are in continuous rotation.

[0044] In order to maintain the electrical continuity of the cylindrical surfaces, the collector module (C) has brushes to remove the nanofibres that remain on the cylindrical surfaces at the end of each fibre production cycle, thus ensuring the continuous electrospinning of the nanofibres on and between the rotating cylindrical surfaces of the collector cylinders.

[0045] The nanofibres electrospun between the cylindrical surfaces of the collector cylinders, in continuous rotation, are deposited on the surface of the deposition table of the deposition module. The table has a substantially circular shape and is positioned between the generatrices of the two collector cylinders, with its surface perpendicular to the electrospinning capillary tube axis.

[0046] In addition, the deposition table has holes extending from its surface to a chamber inside the table, which is connected by a channel to a vacuum pump. The suction force generated by the vacuum, in the holes of the deposition table surface, attaches the deposited nanofibres to the table. The nanofibres, thus fixed to the table, separate from the fixed part, on the cylindrical surfaces of the collector cylinders, by the effect of stretching their cross-section, due to the continuous movement of rotation of the collector cylinders.

[0047] The linear movement of the deposition table, parallel to its surface, and the rotation movement, around its

longitudinal axis, are performed by electric motors controlled by a computerised unit.

[0048] The nanofibres, continuously deposited and attached to the surface of the deposition table, are aligned in different directions by the rotation movement of the deposition table, and the distance between the deposited nanofibres is controlled by the linear movement of the deposition table.

[0049] The continuous deposition of nanofibres, by the cylindrical surfaces of the collector cylinders, allows the formation of a two-dimensional nanofibre mesh of controlled organization and distribution on the surface of the deposition table. The control of the distance between the fibres deposited on the deposition table allows controlling the porosity of the mesh in its plane.

[0050] The controlled movement of the deposition table in the direction and opposite orientation to the electrospinning capillary tube (direction of the matrix thickness) after the formation of the two-dimensional nanofibre mesh on the surface of the deposition table allows the deposition of a new two-dimensional nanofibre mesh over the previous mesh with a new organisation of the nanofibres, the number of nanofibre mesh layers accumulated on the table is defined by the desired thickness of the three-dimensional cell matrix, the thickness of the deposited nanofibres and the magnitude of the vacuum pressure generated on the surface of the deposition table that controls the level of compaction of the nanofibre mesh layers and therefore the porosity of the three-dimensional matrix throughout the thickness.

[0051] The linear movement from the deposition table to the position of the electropulverisation module of the cells occurs after the deposition of one or more layers of two-dimensional nanofibre meshes on the deposition table, the vacuum system being switched off, the electropulverisation module of the cells can basically consist of a syringe to contain a solution with cells in suspension, connected to an injector pump, connected to an electropulverisation capillary tube, which is connected to a voltage source.

[0052] The voltage source is configured to provide positive polarity, being aligned with the ring-shaped collector, with internal diameter equal to the diameter of the deposition table, with negative or neutral polarity.

[0053] The electropulverisation of the cells starts from the capillary tube with positive polarity, in which the solution with cells moves by the action of an electric field towards the collector ring.
The solution with cells is seeded onto the nanofibre meshes deposited on the deposition table, which is in concentric position with the collector ring. In this position, the deposition table starts a rotation movement for a certain period of time, so that the surface of the nanofibre mesh is uniformly distributed with cells.

[0054] The linear movement of the deposition table back to the fibre collector module, followed by a new deposition of another layer or layers of two-dimensional nanofibre meshes, which are successively intercalated in a controlled manner, through the instructions provided by the computerised unit, with the seeding of cells of the desired cell suspension, thus results in a three-dimensional cell matrix with nanofibres of

controlled alignment and uniform cell distribution, throughout the matrix thickness.

[0055] The number of nanofibre mesh layers deposited on the deposition table and the number of times the electropulverisation of the cells occurs is defined by the desired thickness of the three-dimensional cell matrix, as well as by the thickness of the deposited nanofibres, the magnitude of the vacuum pressure generated on the surface of the deposition table that controls the level of compaction of the nanofibre mesh layers and thus the porosity of the three-dimensional matrix throughout the thickness and the desired cell density of the matrix.

[0056] The successive layers of two-dimensional nanofibre meshes, deposited on the surface of the deposition table, are kept in position on the deposition table by the action of the vacuum generated in the holes of the upper surface of the deposition table which communicate with a chamber in its interior connected to the vacuum pump.

[0057] Pressure control in the vacuum pump is also intended to control the degree of compaction between the two-dimensional nanofibre mesh layers formed and thus the porosity in the direction perpendicular to the plane of the deposited nanofibre mesh layer.

[0058] The control of the distance between the electrospinning capillary tube with positive polarity and the collector cylinders, the control of the speed of rotation of the collector cylinders, the control of the linear and rotation movements of the deposition table, the control of the pressure of the vacuum pump, the control of the distance between the

electrospinning capillary tube of the cells with positive polarity and the collector ring, the control of the voltage of the capillary tubes, of the cylindrical surfaces of the collector cylinders and the collector ring are performed by a computerised control unit that, depending on the alignment and distance between nanofibres desired for each deposited two-dimensional layer and on the thickness of the matrix desired and the cell density desired, programs the sequence of all the movements, vacuum pressure and polarity of the electrodes required, based on a computer program developed for this purpose.

[0059] Thus, the system of the present invention has the capacity to continuously and automatically produce three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout their thickness.

[0060] Furthermore, it also provides the possibility of producing matrices with various alignment patterns and with different distances between nanofibres (porosity), throughout the matrix thickness, and with a uniform distribution of tissue component cells, throughout the matrix thickness, in a controlled manner.

[0061] These effects are obtained by combining different technical features, among which we highlight the use of a deposition table with the ability to make controlled linear and rotational movements, thus allowing the possibility of controlling the alignment and the distance between the nanofibres deposited on its surface and the surface of the collection table.

[0062] On the other hand, the surface of the collection table, provided with holes, which are subjected to vacuum pressure, allows the attachment of the fibres to this table, also allowing the control and definition of the degree of compaction (porosity) to be presented between the different layers of deposited two-dimensional nanofibre meshes, as well as the support and deposit of the nanofibres on the deposition table.

[0063] The separation of the deposited fibres, which occurs by the effect of nanofibres stretching on the surface of the collector cylinders, during the continuous movement of these cylinders, and the linear movement of the deposition table in the direction and opposite orientation to the electrospinning capillary tube, contributes advantageously to the deposition of successive layers of two-dimensional fibre meshes, and the linear movement of the deposition table, to the concentric position, with the collector ring of the electropulverisation module of the cells.

[0064] The solution with cells, which is electropulverised over the nanofibre meshes deposited on the deposition table, the rotation movement of the deposition table, in this position, for a certain period of time, ensures the uniform distribution of the cells on the surface of the nanofibre mesh, while the linear movement, returning the deposition table to the fibre collector module, allows a new deposition of another layer or layers of two-dimensional nanofibre meshes.

[0065] The alternating and controlled movement of the deposition table between the nanofibre deposition position and the cell electropulverisation position allows to automatically manufacture a three-dimensional cell matrix with nanofibres of controlled alignment and uniform cell distribution throughout

the thickness, where the number of nanofibre mesh layers deposited on the deposition table and the number of times that the electropulverisation of the cells occurs are defined by the desired thickness of the three-dimensional cell matrix, by the thickness of the deposited nanofibres, by the magnitude of the vacuum pressure generated on the surface of the deposition table that controls the level of compaction of the nanofibre mesh layers and therefore the porosity of the matrix throughout the thickness and by the cell density desired for the matrix.

Detailed description of the invention

[0066] The present invention relates to a system and a process for the automated manufacture of three-dimensional cell matrices.

1. The system of the present invention comprises:

[0067]

a nanofibre formation module (A), comprising a container for containing and delivering molten polymer or polymeric solution, typically a syringe, and an injection pump, connected to an electrospinning capillary tube, connected to a voltage source, configured to provide positive polarity;

a nanofibre collector module (B), for collecting the electrospun nanofibres, comprising two collector cylinders with cylindrical surfaces with negative or neutral polarity, with coaxial axes in continuous rotation movement and exposed to the electrospinning capillary tube;

a nanofibre deposition module (C), for the accumulation and formation of two-dimensional nanofibre meshes collected between the collector cylinders, comprising a deposition table positioned between the generatrices of the collector cylinders with the capacity to perform linear and rotation movements;

an electropulverisation module (D), for electropulverisation of the cells over the two-dimensional nanofibre meshes previously deposited on the deposition table, repeating the seeding of cells alternately with the deposition of successive two-dimensional nanofibre meshes, which comprises a container to contain and provide the solution with cells in suspension, typically a syringe, and an injection pump, connected to an electropulverisation capillary tube, a ring-shaped collector with negative or neutral polarity;
one or more power supplies for the electrospinning and electropulverisation system; and
a device with vacuum producing capability, typically a vacuum pump.

[0068] In addition to the mentioned elements, the system of the present invention also comprises a computerised control unit and the electronics necessary for its proper functioning, actuators, namely the actuators of the linear and rotation movement of the deposition table, as well as all the electric wiring for the distribution of energy to the various components of the system, as well as all the devices and accessories necessary to guarantee the sterilisation, humidity and temperature conditions required to ensure the survival of the cells deposited on the nanofibre meshes during the manufacturing time of the three-dimensional cell matrix.

[0069] More particularly, the system (1) of the present invention comprises:

a nanofibre formation module (A) by electrospinning with an electrospinning capillary tube (4);
a nanofibre collector module (B) with two collection cylinders (6,30);

a nanofibre deposition module (C) of the collected nanofibres, which forms two-dimensional nanofibre meshes with alignment and distance between nanofibres controlled throughout the surface of the deposition table (10) by combination of the linear (22) and rotation (24) movements thereof; and
a cell electropulverisation module (D), where the cells (58) are seeded from the electropulverisation capillary tube (13) over the two-dimensional nanofibre meshes previously deposited on the deposition table (10), repeating the seeding of cells alternately with the deposition of successive layers of two-dimensional nanofibre meshes, thus obtaining a uniform distribution of cells throughout the thickness of the three-dimensional cell matrix;
a vacuum pump (21) for pressure regulation;
[0070] Wherein:
The nanofibre formation module (A) comprises:

an electrospinning capillary tube(4) with positive polarity;
an adjustable length support (3);
[0071] The nanofibre collector module (B) comprises:

two collector cylinders (6, 30) with coaxial, perpendicular and aligned axes with the electrospinning capillary tube axis (4);
two electric motors (7, 29) to move in continuous rotation the collector cylinders (6, 30) controlled by a computerised unit (20), the distance between the top faces of the two collector cylinders (6, 30) is equal to the diameter of the nanofibres deposition table (10);
cylindrical surfaces (34, 36) of the collector cylinders (6, 30) in electrically conductive material with negative or neutral polarity, the electrospun nanofibres (5) are collected

on the cylindrical surfaces and between the cylindrical surfaces (34, 36);
brushes (32, 35) to remove the nanofibres that remain on the cylindrical surfaces (34, 36) maintaining the electrical continuity of the cylindrical surfaces (34, 36) thus ensuring the continued electrospinning of the nanofibres;
[0072] The nanofibre deposition module comprises:

a deposition table (10) of circular shape positioned between the generatrices of the collector cylinders (6, 30) with flat surface perpendicular to the electrospinning capillary tube axis (4), where the electrospun nanofibres (5) between the collector cylinders (6, 30) are deposited by action of the rotation (31) of them;
a chamber (42) inside the table (10) in which the surface of the deposition table with holes (9) extending from its surface to this chamber (42);
a vacuum pump (21) connected to the chamber (42) by a channel (11);
three electric motors (7,39,41) controlled by a computerised unit (20) for moving the deposition table (10) linearly in the direction parallel to its surface (22), towards (27) the electrospinning capillary tube axis (4) and for the rotation movement (24) around its longitudinal axis; and
[0073] The cell electropulverisation module (D) comprises:

a container for containing and delivering a solution with cells in suspension (14), typically a syringe, and an injection pump, connected to an electropulverisation capillary tube (13);
a voltage source configured to provide positive polarity connected to the electropulverisation capillary tube (13) ;
an adjustable length support (15),

a ring-shaped collector (12) with the same internal diameter as the diameter of the deposition table (10), this ring having either negative or neutral polarity (18) ;

whereby:

the control of the linear (22) and rotation (24) movements of the deposition table (10) when positioned between the generatrix of the collector cylinders (6, 30) allows the two-dimensional organisation of the deposited nanofibre mesh allowing the control of its alignment (81,82,83,84) on the surface of the deposition table (10) as well as the distance (45, 47, 49, 52) between the deposited nanofibres;

the deposition table (10) presents a controlled movement (27, 60), towards the capillary tube axis (4), allowing this movement to deposit layers of two-dimensional nanofibre meshes over the previously deposited meshes;

the vacuum pump (21) exerts a controlled vacuum pressure on the holes of the deposition table (9), allowing nanofibres to be attached to the surface of the deposition table (10);

the deposition table (10) moves alternately (55, 59) between the position of the nanofibre collector module where the nanofibres are deposited and the electropulverisation module where the cells (58) are seeded (56) over the two-dimensional nanofibre meshes;

the number of nanofibre mesh layers deposited (65, 64, 66) over the deposition table (10) and the number of times the electropulverisation of the cells occurs is defined by the desired thickness (67) of the three-dimensional matrix (68), the thickness of the nanofibres, the magnitude of the vacuum pressure that controls the level of compaction of the nanofibre mesh layers and thus the porosity of the matrix throughout the thickness and the desired cell density of the matrix.

[0074] The nanofibre (5) of polymeric material, formed by electrospinning from the capillary tube (4), with positive

polarity, moves by action of an electric field towards a collector module, which is composed of two collector cylinders (6, 30) with coaxial axes perpendicular and aligned with the axis of the electrospinning capillary tube (4), each cylinder is provided with continuous rotation movement (31), by an electric motor (7, 29) controlled by a computerised unit (20), the distance between the top faces of the two cylinders (6, 30) is equal to the diameter of the nanofibres deposition table (10).

[0075] The cylindrical surfaces of the collector cylinders (34, 36)) are made of conductive material, and these can have negative or neutral polarity (33).

[0076] The electrospun nanofibres (5) are collected on the cylindrical surfaces (34, 36) and between the cylindrical surfaces (34, 36).

[0077] Module (B) has brushes (32, 35) to remove the nanofibres that remain on the cylindrical surfaces (34,36) maintaining the electrical continuity of these surfaces (34,36) thus ensuring the continued electrospinning of the nanofibres on and between the cylindrical surfaces in rotation (31).

[0078] The nanofibres deposition module (C) comprises a deposition table (10), of circular shape, positioned between the generatrices of the collector cylinders (6, 30), with a flat surface perpendicular to the axis of the electrospinning capillary tube (4), where the nanofibres, electrified between the collector cylinders (6, 30), are deposited by rotation action (31) of them.

[0079] The deposition table (10) has a surface with holes (9), extending to a chamber (42), which is located inside the table (10), this chamber (42) being connected by a channel (11) to a vacuum pump (21).

[0080] The deposited nanofibres (8) are attached to the deposition table (10) by action of the suction force (43), generated by the vacuum in the holes of the surface of the deposition table (10). These nanofibres (8), attached to the table (10), separate from their fixed part on the cylindrical surfaces (34,36) of the collector cylinders (6,30) by a stretching effect of their cross-section, due to the continuous rotation movement (31) of the collector cylinders (6,30).

[0081] The deposition table (10) moves linearly parallel to its surface (22) and has a rotation movement (24) around its longitudinal axis, the linear and rotation movements of the table are performed by electric motors (39, 40) controlled by a computerised unit (20), the control of these movements allow defining the two-dimensional organisation of the nanofibre mesh deposited on the deposition table (81, 82, 83, 84) .

[0082] The continuously deposited nanofibres (8) attached to the surface (9) of the deposition table (10) are aligned in different directions by the rotation movement of the deposition table (24). The distance between the deposited nanofibres is controlled by the linear movement of the deposition table (22). The continuous deposition of nanofibres, by the cylindrical collector surfaces (34, 36), allows the formation of a two-dimensional nanofibre mesh (81, 82, 83, 84), of controlled organisation and distribution, over the surface of the deposition table (10). The control of the distance (45, 47, 49, 52) between the nanofibres deposited on the deposition

table (10) allows controlling the porosity of the two-dimensional mesh on the plane thereof.

[0083] After the two-dimensional nanofibre meshes (81, 82, 83, 84) is formed over the surface of the deposition table (10), the linear movement of the deposition table in the direction and opposite orientation (60) to the electrospinning capillary tube (4) determines the end of an electrospinning cycle.

[0084] Thus, it is possible to start a new deposition of two-dimensional nanofibre meshes (82) over the previously formed meshes (81), with a new organization of the nanofibres.

[0085] The number of layers (64, 65, 66) of nanofibre meshes deposited onto the table is defined by the desired thickness (67) for each three-dimensional cell matrix (68), the thickness of the deposited nanofibres and the magnitude of the vacuum pressure generated on the surface of the deposition table (10) which controls the level of compaction of the nanofibre mesh layers and hence the porosity of the three-dimensional matrix (68) throughout the thickness (67).

[0086] The cell electropulverisation module (D) comprises a container (14), for containing and delivering a solution with cells in suspension, typically a syringe, and an injection pump, connected to an electropulverisation capillary tube (13), connected to a voltage source (17), configured to provide positive polarity (16), an adjustable length support (15), a ring-shaped collector (12) with an internal diameter identical to the diameter of the deposition table (10), this ring having negative or neutral polarity (18).

[0087] After deposition of one or more mesh layers (81,82,83,84) of nanofibres over the deposition table (10), it moves linearly (55) towards the cell electropulverisation module (D), until it is centred with the ring-shaped collector (12), which presents negative or neutral polarity (18). In this position, the deposition table (10) starts a rotation movement (57) on its axis and the vacuum system is turned off, initiating the seeding of the cells (56) from the electropulverisation capillary tube (13), with positive polarity (16), on the nanofibre meshes (81,82,83,84), during a certain period of time, thus leaving the surface of the nanofibre mesh with cells (58) uniformly distributed.

[0088] After the electropulverisation of the cells (56), over the layers of nanofibre meshes (81,82,83,84), the deposition table (10) moves (59) again to the fibre collector module, in order to proceed to a new deposition cycle of another layer or layers of two-dimensional nanofibre meshes with controlled orientation and distance between nanofibres.

[0089] The deposition table (10) is alternately moved (55, 59) between the position of the nanofibre collector module, where the nanofibres are deposited, and the electropulverisation module, where the cells (56) are seeded in a controlled manner by the computerised unit (20), thus obtaining a three-dimensional cell matrix (68) with nanofibres of controlled alignment and uniform cell distribution throughout the thickness (67).

[0090] The number of nanofibre mesh layers deposited (64,65,66) over the deposition table (10) and the number of times the electropulverisation of the cells (56) occurs over these is defined by the thickness (67) desired for the three-dimensional

cell matrix (68), by the thickness of the deposited nanofibres, the magnitude of the vacuum pressure generated at the surface of the deposition table that controls the level of compaction of the nanofibre mesh layers and thus the porosity of the three-dimensional matrix throughout the thickness and the cell density desired for the three-dimensional cell matrix (68).

[0091] The successive two-dimensional layers of nanofibre meshes, deposited (81,82,83,84) onto the deposition table (10), are held in position by the action of a suction force (43) generated by the vacuum pressure in the holes of the surface (9) of the deposition table (10), which communicate with a chamber (42), in its interior, connected by a channel (11) to the vacuum pump (21).

[0092] The control of the pressure in the vacuum pump (21) is also intended to control the suction forces (43) on the fibres and the degree of compaction between the formed two-dimensional nanofibre mesh layers (81,82,83,84) and, consequently, the porosity in the direction perpendicular to the plane of the deposited fibre layer.

[0093] The control of the distance between the electropulverisation capillary tube (4) with positive polarity and the generatrix of the collector cylinders (6, 30), the control of the rotation speed (31) of the collector cylinders (6, 30), the control of the linear (22) and rotation movements (24) of the deposition table, control of the pressure of the vacuum pump (21), control of the distance between the electropulverisation capillary tube (13) of the cells with positive polarity and the collector ring (12), control of the voltage on the capillary tubes (38) and the cylindrical surfaces (34, 36) of the collector cylinders (6, 30) and the

collector ring (12) is performed by a computerised control unit (20), which, depending on the desired alignment and distance of the nanofibres for each deposited two-dimensional nanofibre mesh layer (81, 82, 83, 84), the desired thickness (67) of the matrix (68) and the desired cell density, programmes the sequence of all the necessary movements, vacuum pressure and polarity of the electrodes on the basis of a computer programme specifically developed for this purpose.

2. Automated manufacturing process for three-dimensional cell matrices with nanofibres with controlled alignment and uniform cell distribution throughout the thickness

[0094] The process of the present invention is carried out in several steps using the manufacturing system (1), as described in the previous section.

[0095] The production of three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness occurs from the alternating linear movement (55, 59) of the deposition table (10) between the position of the nanofibre collector module, where the aligned nanofibres (8) are deposited, and the electropulverisation module, where the cells (56) are seeded onto the two-dimensional nanofibre mesh layers (81,82,83,84).

[0096] The continuously deposited nanofibres (8) attached to the surface of the deposition table (10) are aligned in different directions by the rotation movement of the deposition table (24), the distance between the deposited nanofibres is controlled by the linear movement (22) of the deposition table (10), the continuous deposition of nanofibres by the cylindrical surfaces (34, 36) of the collector cylinders (6, 30) allows the formation of two-dimensional nanofibre meshes

(81,82,83,84) of controlled organization and distribution on the surface (9) of the deposition table (10), the control of the distance (45, 47, 49, 52) between the fibres deposited on the deposition table (10) allows controlling the porosity of the two-dimensional mesh in the plane thereof;

[0097] The controlled linear movement of the deposition table in the direction and opposite orientation (60) to the electrospinning capillary tube (4) allows the deposition of a new two-dimensional nanofibre mesh over the previous mesh with a new organisation of the nanofibres, being the number of layers (64,65, 66) of nanofibre meshes deposited on the table (10) defined by the desired thickness (67) of the three-dimensional cell matrix (68), the thickness of the deposited nanofibres and the magnitude of the vacuum pressure generated on the surface of the deposition table (9) which controls the level of compaction of the nanofibre mesh layers and thus the porosity of the three-dimensional matrix throughout the thickness (67).

[0098] Thus, the process for manufacturing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness of the present invention comprises the following steps:

a) exposure of the two cylindrical surfaces (34, 36) of the collector cylinders (6, 30) to the electrospinning capillary tube (4) containing a solution of a given polymer suited to the matrix to be produced, such exposure being made by applying a negative or neutral voltage to the two cylindrical surfaces (34, 36), the collector cylinders (6, 30) being induced with a permanent rotation movement (31) for the continuous electrospinning of nanofibres (5) over these surfaces and

between these surfaces (34, 36) in the area of the generatrix closest to the electrospinning capillary tube (4);

b) continuous deposition of the electro-spun nanofibres (5) between cylindrical surfaces (34,36) over the surface with holes (9) of the deposition table (10), positioned between the generatrices of the collector cylinders (6,30), by the action of the continuous rotation movement (31) of the collector cylinders (6,30);

c) application of vacuum pressure to the deposited nanofibres from the deposition table (10) through holes in its surface (9) to attach and compact the nanofibres to the deposition table (10);

d) linear (22) and rotation (24) movements of the deposition table (10) in order to align and space (44, 45, 47, 49, 52) the nanofibres deposited by the cylindrical surfaces (34,36) forming a two-dimensional nanofibre mesh (81) of controlled organisation and distribution over the surface (9) of the deposition table (10);

e) rupture of the nanofibres attached to the deposition table (10) by the effect of stretching their cross-section due to the continuous rotation movement (31) of the collector cylinders (6,30);

f) linear movement (22) of the deposition table (10) in the direction and opposite orientation (60) to the electrospinning capillary tube (4);

g) repetition of the cycles as described in step (d), (c) and (f) as often as necessary in order to deposit successive layers of two-dimensional nanofibre meshes (81,82,83,84) on the meshes deposited in the previous cycle;

h) disruption of the fibre electrospinning process;

i) linear movement (55) of the deposition table (10) to the concentric position with the ring collector (12) of the cell electropulverisation module;

j) stopping the application of vacuum pressure to the surface of the deposition table (9);

k) exposure of the two-dimensional nanofibre meshes (81,82,83,84) on the deposition table (10) to the electropulverisation capillary tube (13) containing a solution of a given medium with cells in suspension, this exposure being done by applying a negative or neutral voltage (18) to the collector ring (12) around the deposition table (10) for a period of time;

l) rotation movement (57) of the two-dimensional nanofibre meshes (81,82,83,84) by action of the rotation of the deposition table (10) for uniform cell distribution (58) on the surface of the nanofibre meshes;

m) stopping the electropulverisation of the cells;

n) application of the vacuum pressure to the surface of the deposition table (10);

o) linear movement (59) of the deposition table (10) to the position between the generatrices of the collecting cylinders (6,30);

p) repetition of cycles, as described in steps (a) to (o), as many times as necessary, the factors relating to rotation (24) and linear movement of the deposition table (22) in step (d) can be modified with respect to the previous cycle, to form two-dimensional nanofibre meshes with different alignment and distance between nanofibres than those obtained in the previous cycle.

[0099] Accordingly, the controlled movement (27) of the deposition table (10) in the direction and opposite orientation (60) to the electrospinning capillary tube (4) after a set of layers of deposited fibre meshes followed by electropulverisation of the cells (56) allows the accumulation of successive layers of two-dimensional nanofibre meshes (81,82,83, 84) with cells (58) and the formation of a three-

dimensional cell matrix (68) with nanofibres of controlled alignment and uniform cell distribution with thickness (67) dependent on the number of layers (64,65,66) of deposited fibres, the thickness of the nanofibres and the degree of compaction between layers which is controlled by vacuum pressure and the vacuum pressure attaches the fibres to the table.

[0100] The process of manufacturing three-dimensional cell matrices (68) with nanofibres of controlled alignment and uniform cell distribution throughout the thickness (67) takes place continuously by successively performing the various steps for the formation of two-dimensional layers of nanofibres and cell electropulverisation, according to the above.

3. Characterisation of three-dimensional matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness

[0101] The obtained matrix thickness varies according to the number of deposited layers of nanofibre meshes, the thickness of these nanofibres and the degree of compaction between layers, the latter controlled by the vacuum pressure exerted on the layers of fibres deposited on the surface of the deposition table.

[0102] On the other hand, the alignment and distance, between the nanofibres in each layer is controlled by the different rotation and linear movements of the deposition table in each fibre deposition step.

[0103] The cell density of the three-dimensional nanofibre matrix is defined by the number of deposited nanofibre layers, the thickness of the deposited nanofibres, the magnitude of

the vacuum pressure generated at the surface of the deposition table that controls the level of compaction of the nanofibre mesh layers, the number of times the electropulverisation of the cells (56) over the two-dimensional nanofibre mesh occurs and the duration of the cell electropulverisation period on the meshes.

[0104] In conclusion, by implementing the system and process of the present invention it is possible to manufacture three-dimensional cell matrices (68) with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that occurs from the alternated linear movement of the deposition table between the position of the nanofibre collector module where the aligned nanofibres are deposited and the electropulverisation module where the cells are seeded on the two-dimensional nanofibre mesh layers, which can present several alignment patterns and distance between nanofibres, throughout the thickness of the matrix, this thickness also being variable.

4. Applicability of matrices

[0105] Example: Production of a three-dimensional cell matrix with nanofibres of controlled alignment and uniform cell distribution throughout the thickness.

[0106] This example concerns the production of a cell matrix composed of 48 layers of aligned polymeric nanofibre meshes and cells from a chondrocyte cell line for cartilage engineering, with a total thickness of 3 mm.

[0107] The polymer that was used to make the matrix was polycaprolactone (PCL) with a molecular weight of 80,000 Da. PCL was dissolved with a 12% concentration of dichloromethane

(DCM) and dimethylformamide (DMF) at a ratio of 1:1 (v:v) after being stirred for 12 hours at room temperature.

[0108] The molten polymer was then electrospun using a capillary tube (4) with a flow of 2.5 mL/h, a voltage of 25 kV and a working distance of 15 cm from the cylindrical surfaces (34,36) of the collector cylinders (6,30).

[0109] In this configuration of the electrospinning system (1), the collector cylinders (6,30) have a diameter of 80 mm and are moved with a continuous rotational speed of 10 rpm and the cylindrical surfaces (34,36) were subjected to a negative voltage of -3 kV.

[0110] The deposition table has a diameter of 6 mm, and the holes on its surface (9) are subjected to a vacuum pressure of 3300 Pa. For the formation of each two-dimensional nanofibre mesh layer with different alignments and distance between nanofibres, different combinations of speeds and duty strokes were programmed in linear movement (22) and rotation movement (24) of the deposition table (10).

[0111] The 48 layers of the deposited two-dimensional nanofibre meshes resulted from performing 6 consecutive cycles (6 times) of 8 layers of two-dimensional meshes with different alignments (81), (82), (83), (84), (85), (86), (87) and (88) in the following order, with the speed and stroke characteristics of the deposition table (10) for each mesh as follows:

two-dimensional mesh (81) obtained with linear stroke (46) from -1.5 mm to -0.7 mm at a linear speed of 3 m/s and an angular stroke of +0°;

two-dimensional mesh (82) obtained with linear stroke (48) from 0.1 mm to -0.8 mm at a linear speed of 6 m/s and an angular stroke (49) of +22.5°;
two-dimensional mesh (83) obtained with a linear stroke (50) from 0 mm to +0.7 mm at a linear speed of 1 m/s and an angular stroke (51) of 40°;
two-dimensional mesh (84) obtained with linear stroke (53) from +0.8 mm to +2 mm at a linear speed of 2 m/s and an angular stroke (54) of 50°;
two-dimensional mesh (85) obtained with linear stroke of 1.5 mm to -0.7 mm at a linear speed of 1 m/s and an angular stroke of +0°;
two-dimensional mesh (86) obtained with linear stroke from 0.1 mm to -0.8 mm at a linear speed of 3 m/s and an angular stroke of -22.5°;
two-dimensional mesh (87) obtained with linear stroke from 0mm to +0.7mm at a linear speed of 5 m/s and an angular stroke of -40°;
two-dimensional mesh (88) obtained with linear stroke from +0.8mm to +2mm at a linear speed of 3 m/s and an angular stroke of -50°;

[0112] For the cell electropulverisation process an immortalised human chondrocyte cell line C28 / I2 was used and maintained at 37°C in a humidified atmosphere of 5% CO2 in air, in DMEM / F-12 (Sigma - Aldrich) supplemented with 10% (v / v) of FBS (Sigma -Aldrich), 1% (v / v) P / S (Sigma - Aldrich) and 0.25 µg / mL of amphotericin B. Cells were harvested pre-confluence using a trypsin / EDTA (0.25%; Sigma-Aldrich) solution. 1.00 × 10^6 chondrocytes were suspended in 154 µL of culture medium and poured into a 5 mL plastic syringe.

[0113] The chondrocyte suspension was subjected to electropulverisation with a flow rate of 2 mL / h at +12.5 kV

through 27G capillary tube/blind needle (13) (0.4 mm diameter and 1.5 mm length) with a distance between the needle and the ring-shaped collector (12) of 70 mm, the deposition table (10) was positioned concentrically with the collector ring (12). The collector ring was subjected to a voltage of -1 kV. In this position the deposition table (10) started the rotation movement (57) at a speed of 5 rpm for 3s.

[0114] At the end of each four layers of two-dimensional nanofibres mesh deposited on the deposition table (10), it moved 0.0625 mm in the direction and opposite orientation (60) to the electrospinning capillary tube (4), which corresponds to the average thickness of the four layers of deposited two-dimensional nanofibres mesh. In total, the deposition table moved in the opposite orientation of the capillary tube about 3 mm, corresponding to the thickness of the matrix (67) obtained at the end of the 48 deposited layers.

[0115] After every four deposited mesh layers (81, 82, 83, 84) the deposition table moves (55) to the position of the cell electropulverisation module, the vacuum on the deposition table is turned off and the cells (58) are seeded on the meshes, then the deposition table (10) moves (59) to the nanofibre collector module for new deposition of four layers of nanofibre meshes (85, 86, 87, 88), the vacuum system is turned on again. This automated and alternated process between the deposition of the nanofibre meshes and the seeding of cells (58) on them was repeated until the final thickness (67) of the aligned nanofibre cell matrix was reached (65).

[0116] The three-dimensional matrix of aligned fibres obtained in this example exhibits, like native cartilage, a preferential alignment of the fibres in its surface area parallel to the

surface, in the intermediate area it does not show any preferential alignment, and in the deeper area the fibres are aligned in a vertical manner relative to the surface.

Claims

1. An automated manufacturing system for three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness comprising (a) a nanofibre formation module by electrospinning, (b) a nanofibre collector module, (c) a deposition module of the collected nanofibres, which forms two-dimensional nanofibre meshes (81,82,83,84) with alignment and distance between nanofibres controlled by a combination of linear (22) and rotation movement (24) of the deposition table (10), these meshes accumulate in successive layers forming the thickness of the cell matrix (67), the surface with holes (9) of the deposition table (10) is connected by a channel to a vacuum pump (21), (d) a cell electropulverisation module, wherein the linear movement (55) of the deposition table (10) from the position in the nanofibre collector module to the cell electropulverisation position (56) seeds the cells of the nanofibre meshes previously deposited on the deposition table (10) by repeating the electropulverisation of cells in an alternated manner with the deposition of successive two-dimensional nanofibre meshes thus obtaining a uniform distribution of cells throughout the thickness of the three-dimensional cell matrix, characterised in that:

a. The nanofibre formation module comprises an electrospinning capillary tube (4) with positive polarity and an adjustable length support (3);

b. The nanofibres collector module comprises two collector cylinders (6,30) with coaxial and perpendicular axes to the electrospinning capillary tube axis (4), each cylinder is provided with continuous rotation movement (31) through an electric motor (7,29) controlled by a computerised unit (20), the distance between the top faces of the two collector cylinders (6,30) is equal to the diameter of the nanofibres deposition table (10), the cylindrical surfaces (34, 36) are made of conductive material with negative or neutral polarity (33), the electrospun nanofibres (5) are collected on the cylindrical surfaces (34,36) and between the cylindrical surfaces (34,36), this module is provided with brushes (32, 35) to remove the nanofibres that remain on the cylindrical surfaces (34,36) maintaining the electrical continuity of the cylindrical surfaces (34,36) thus ensuring the continuous electrospinning of the nanofibres on and between the rotating (31) cylindrical surfaces (34,36);

c. The nanofibre deposition module comprise a deposition table (10) in a circular shape positioned between the generatrices of the collector cylinders (6, 30) having a surface with holes (9) perpendicular to the electrospinning capillary tube axis (4) where the electrospun nanofibres between the collector cylinders (6, 30) are deposited by action of rotation (31) of these, the deposition table (10) has holes extending from its surface (9) to a chamber (42) which is inside the deposition table (10), this chamber being connected, by a channel (11), to a vacuum pump (21), the deposition table (10) moves linearly (22) parallel to its surface (9) and towards (27) the electrospinning capillary tube axis (4), the deposition table (10) has rotation movement (24) around its longitudinal axis, the linear (22, 27) and rotation (24) movements of the deposition table (10) are performed by electric motors (39,

40,41) controlled by a computerised unit (20), the control of these movements allows the two-dimensional organization of the nanofibre mesh (81,82,83,84) allowing the control of its alignment over the surface of the deposition table (10) as well as the distance (44,45,47,49,52) between the deposited nanofibres;

d. The cell electropulverisation module comprises a container (14) for containing and supplying a solution with cells in suspension, typically a syringe, and an injector pump, connected to an electropulverisation capillary tube (13), connected to a voltage source (17), configured to provide positive polarity (16), an adjustable length support(15), a ring-shaped collector (12) with internal diameter identical to the diameter of the deposition table (10) with this ring having negative or neutral polarity (18), the deposition table (10) alternately moves (55,59) between the position of the nanofibre collector module where the nanofibres are deposited and the electropulverisation module where the cells (56) are seeded on the two-dimensional nanofibre mesh layers (81,82,83,84);
wherein:

- the cylindrical surfaces (34,36) with negative or neutral polarity (33), collect the nanofibres (5) from the electrospinning capillary tube (4) with positive polarity (38) in a continuous manner by the action of the rotation movement (31) of the collector cylinders (6,30);

- through the rotation movement (31) of the collector cylinders (6, 30) controlled by the computerised unit (20), the electrospun nanofibres (5) between the cylindrical surfaces (34, 36) are deposited (8) on the surface (9) of the deposition table (10) being the nanofibres attached to the deposition

table (10) by action of the suction force (43) generated by the vacuum in the holes of the surface (9) of the deposition table (10), these nanofibres (8) attached to the deposition table (10) separate from their fixed part on the cylindrical surfaces (34, 36) by a stretching effect of their cross-section, due to the continuous rotation movement (31) of the collector cylinders (6,30);

- the continuously deposited nanofibres which are attached to the surface (9) of the deposition table (10) are aligned in different directions (81,82,83,84) by the rotation movement (24) of the deposition table (10), the distance (44,45,47,49,52) between the deposited nanofibres is controlled by the linear movement (22) of the deposition table (10), all movements are controlled by a computerised unit (20), the continuous deposition of nanofibres (8) on the cylindrical collector surfaces (34,36) allows the formation of two-dimensional nanofibre meshes (81,82,83,84) of controlled organisation and distribution over the surface (9) of the deposition table (10), the control of the distance (44,45,47,49,52) between the deposited nanofibres on the deposition table (10) allows to control the porosity of the two-dimensional mesh in the plane thereof;

- after the formation of one or several two-dimensional meshes (81,82,83,84) of nanofibres over the surface (9) of the deposition table (10), the linear movement (27) of the deposition table (10) in the direction and opposite orientation (60) to the electrospinning capillary tube (4) allows the deposition of new two-dimensional nanofibre meshes over the previous meshes, the number of layers (64,65, 66) of nanofibre meshes deposited onto the table being defined by the desired thickness (67) of the three-dimensional cell matrix (68), the

thickness of the deposited nanofibres and the magnitude of the vacuum pressure generated on the surface (9) of the deposition table (10) which controls the level of compaction of the layers of nanofibre meshes and thus the porosity of the three-dimensional matrix throughout the thickness (67) ;

- after the deposition of one or more layers of nanofibre meshes on the deposition table (10) it moves linearly (55) to the cell electropulverisation module until it is concentric with the ring-shaped collector (12), which has negative or neutral polarity (18), in this position the deposition table (10) starts the rotation movement (57) about its axis and the vacuum system is turned off, starting the electropulverisation of cells (56) from the capillary tube (13) with positive polarity (16) over the nanofibre meshes (81,82,83,84) for a period of time defined in function of the desired cell density, thus getting the nanofibres meshes seeded with cells (58) uniformly distributed, after seeding the cells the deposition table (10) moves (59) to the fibre collector module in order to proceed to a new deposition of one or more layers of two-dimensional nanofibre meshes with controlled orientation and distance between nanofibres, successively intercalating in a controlled way by the computerised unit (20) the deposition of nanofibre meshes (81,82,83,84) and the seeding of cells (56), thus manufacturing a three-dimensional cell matrix (68) with nanofibres of controlled alignment and uniform cell distribution throughout the thickness (67) .

- the number of layers (64,65,66) of nanofibre meshes deposited on the deposition table (10) and the number of times the cell electropulverisation (56) occurs over them is defined by the desired thickness (67) of the three-dimensional cell matrix (68), by the thickness of the deposited nanofibres, the

magnitude of the vacuum pressure generated at the surface of the deposition table (10) which controls the level of compaction of the nanofibre mesh layers and hence the porosity of the three-dimensional matrix throughout the thickness (67) and the desired cell density of the three-dimensional cell matrix.

2. Manufacturing system (1) according to the preceding claim comprising computerised control unit (20) and computer programme.

3. An automated process for producing three-dimensional cell matrices with controlled alignment and uniform cell distribution nanofibres throughout the thickness that takes place in the system of any of claims 1 or 2 characterised in that it comprises the following steps:

a. exposure of the two cylindrical surfaces (34,36) of the collector cylinders (6,30) to the electrospinning capillary tube (4) containing a solution of a given polymer suitable for the function of the matrix to be produced, such exposure being made by applying a negative or neutral voltage to the two cylindrical surfaces (34, 36) being the collector cylinders (6, 30) animated by rotation movement (31) for the continuous electrospinning of nanofibres (5) on these and between these surfaces (34, 36) in the area of the generatrix closest to the electrospinning capillary tube (4);

b. continuous deposition of the electrospun nanofibres between cylindrical surfaces (34,36) over the surface with holes (9) of the deposition table (10), positioned between the generatrices of the collector cylinders (6,30), by action of

the continuous rotation movement (31) of the collector cylinders (6,30);

c. vacuum pressure application to the deposited nanofibres from the deposition table (10) obtained through holes in its surface (9) to attach and compact the nanofibres to the deposition table (10);

d. linear (22) and rotation (24) movements of the deposition table (10) to align and space the nanofibres deposited by the cylindrical surfaces (34,36), forming a two-dimensional nanofibre mesh (81,82,83,84) of controlled organisation and distribution over the surface of the deposition table (10);

e. rupture of the nanofibres (8) attached to the deposition table (10) by the effect of stretching its cross-section, by action of the rotation movement (31) of the collector cylinders (6,30);

f. linear movement (27) of the deposition table (10) in the direction and opposite orientation (60) to the electrospinning capillary tube (4);

g. repetition of cycles as described in step (d), (c) and (f) as often as necessary in order to deposit successive layers of two-dimensional nanofibre meshes over the meshes deposited in the previous cycle;

h. disruption of the fibre electrospinning process;

i. linear movement (55) of the deposition table (10) to the concentric position with the ring collector (12) of the cell electropulverisation module;

j. interruption of the application of vacuum pressure on the surface of the deposition table (10) ;

k. exposure of the two-dimensional nanofibre meshes (81,82,83,84) on the deposition table (10) to the electropulverisation capillary tube (13) containing a solution of a given medium with cells in suspension, this exposure being done by applying a negative or neutral voltage (18) to the collector ring (12) around the deposition table (10) for a period of time;

l. rotation movement (57) of the deposition table (10) for a period of time for uniform cell seeding and control of cell density in the plane of the deposited nanofibre meshes (81,82,83,84) on the deposition table (10);

m. stopping the electropulverisation of cells (56);

n. application of vacuum pressure to the surface of the deposition table (10);

o. linear movement (59) of the deposition table (10) to the position between the generatrices of the collecting cylinders (6,30);

p. repetition of cycles, as described in steps (a) to (o), as many times as necessary, the parameters of rotation (24) and linear movement (22) of the deposition table (10) in step (d) can be modified with respect to the previous cycle, to form two-dimensional nanofibre meshes with alignment and distance between nanofibres (81,82,83,84) that are different from those obtained in the previous cycle;

wherein:
the controlled movement (27) of the deposition table (10) in the direction and opposite orientation (60) to the electrospinning capillary tube (4) after each layer of deposited fibres followed by electrospinning of the cells allows the accumulation of successive layers of two-dimensional nanofibre meshes (81,82,83, 84) with cells (58) and the formation of a three-dimensional cell matrix (68) with nanofibres of controlled alignment and controlled cell distribution with thickness (67) dependent on the number of layers (64,65,66) of deposited fibres, the thickness of the nanofibres and the degree of compaction between layers which is controlled by vacuum pressure and the same vacuum pressure attaches the fibres to the table.

4. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness which, according to the preceding claim, occurs in the system of any of claims 1 or 2, characterised in that the collector cylinders (6,30) have cylindrical conductive surfaces (34, 36) with a continuous rotation movement (31) with these surfaces being continuously exposed to the electrospinning capillary tube (4), the continuity of the electrical conductivity of the cylindrical surfaces (34, 36) being ensured by the uninterrupted removal of the nanofibres deposited on these surfaces by cleaning brushes (32, 35) in permanent contact with these surfaces in the generatrix area of the collector cylinders (6,30).

5. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness which, according to any one of claims 3 or 4, occurs in the system of any one of

claims 1 or 2, characterised in that the deposition table (10) continuously accumulates the electrospun nanofibres between the cylindrical surfaces (34, 36) of the collector cylinders (6, 30), said deposition table (10) integrating holes extending from its surface (9) to a cavity in its interior (42) this cavity being connected (11) to a vacuum pump (21) with pressure control, wherein the deposition table (10) has linear movement (27) controlled towards the electrospinning capillary tube axis (4).

6. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any one of claims 3 to 5, occurs in the system of any one of claims 1 or 2, characterised in that the alignment and distance between the deposited nanofibres on the surface of the deposition table (9) is performed by the combination of rotation (24) and linear (22) movements of the deposition table (10) forming a two-dimensional nanofibre mesh (81,82,83,84) of controlled organisation and distribution, the porosity in the plane of the two-dimensional mesh of deposited nanofibres being controlled by the distance (44,45,47,49,52) between the deposited nanofibres.

7. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any one of claims 3 to 6, occurs in the system of any one of claims 1 or 2, characterised in that the suction force (43) generated by the vacuum in the holes of the surface (9) of the deposition table (10) attaches the nanofibres to the deposition table (10) separating these nanofibres from the part still attached to the cylindrical surfaces (34,36) by the effect of

stretching their cross-section, by action of the continuous rotation movement (31) of the collector cylinders (6,30).

8. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any one of claims 3 to 7, occurs in the system of any one of claims 1 or 2, characterised in that the linear movement of the deposition table in the direction and opposite orientation (60) to the electrospinning capillary tube (4) allows successive layers of two-dimensional nanofibre meshes (81, 82,83,84) over the deposition table (10) to form a three-dimensional cell matrix structure (68) of fibres in which its thickness (67) is dependent on the number of layers (64,65,66) of deposited two-dimensional fibres, the thickness of the fibres and the degree of compaction between layers desired by action of the system and vacuum pressure.

9. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any of claims 3 to 8, occurs in the system of any of claims 1 or 2, characterised in that the pressure control in the vacuum pump (21) controls the degree of compaction between the two-dimensional nanofibre layers (81,82,83,84) formed over the deposition table (10) and the porosity in the direction perpendicular to the plane of the deposited nanofibre layer.

10. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any of claims 3 to 9, occurs in the system of any of claims 1 or 2, characterised in that the various parameters of speed,

position, voltage and vacuum are controlled by computerised control unit (20) and one by computer program.

11. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any of claims 3 to 10, occurs in the system of any of claims 1 or 2, characterised in that the linear movement of the deposition table (55) from the generatrix position of the collector cylinders (6, 30) to the position of the cell electropulverisation capillary tube centred on the collector ring (12) allows seeding of the cells onto the two-dimensional nanofibre meshes (81,82,83,84) previously deposited on the surface of the deposition table (10).

12. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any one of claims 3 to 11, occurs in the system of any one of claims 1 or 2, characterised in that at the end of the movement of the deposition table (55) to the position of the cell electropulverisation capillary tube, the application of vacuum pressure to the surface of the deposition table (9) is interrupted preventing suction of the cell suspension medium, the vacuum pressure being applied again to the surface of the deposition table (9) at the start of the linear movement (59) to the position of deposition of the nanofibres by the collector cylinders (6,30).

13. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any one of claims 3 to 12, occurs in the system of any one of

claims 1 or 2, characterised in that the rotation movement (57) of the deposition table (10) for a period of time, when the latter is in the centred position with the collector ring (12) and cell electropulverisation capillary tube (13), allows for uniform cell seeding and cell density control in the plane of the deposited nanofibre meshes (81,82,83,84) on the deposition table (10);

14. The automated process for producing three-dimensional cell matrices with nanofibres of controlled alignment and uniform cell distribution throughout the thickness that according to any one of claims 3 to 13, occurs in the system of any of claims 1 or 2, characterised in that the alternating linear movements (55,59) of the deposition table (10) between the generatrix position of the collector cylinders (6, 39) for deposition of the nanofibre meshes (81,82,83,84) and the position of the cell electropulverisation capillary tube (13) allows alternating deposition of the nanofibre meshes and cell seeding (56) thus manufacturing a three-dimensional cell matrix (68) with nanofibres of controlled alignment and uniform cell distribution throughout the thickness (67).

15. Three-dimensional matrices that occur in the system of any of claims 1 or 2 and are produced according to any one of claims 3 to 14, characterised in that they have applications in medicine, regenerative medicine and/or cartilage engineering.

Drawings

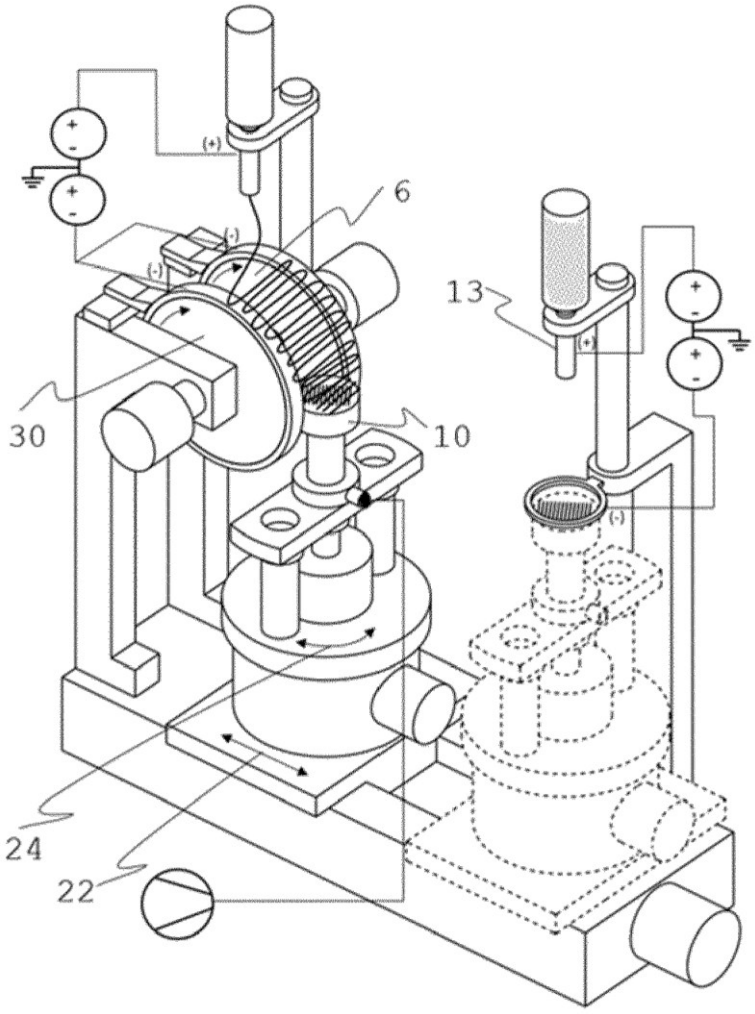

Fig. 1

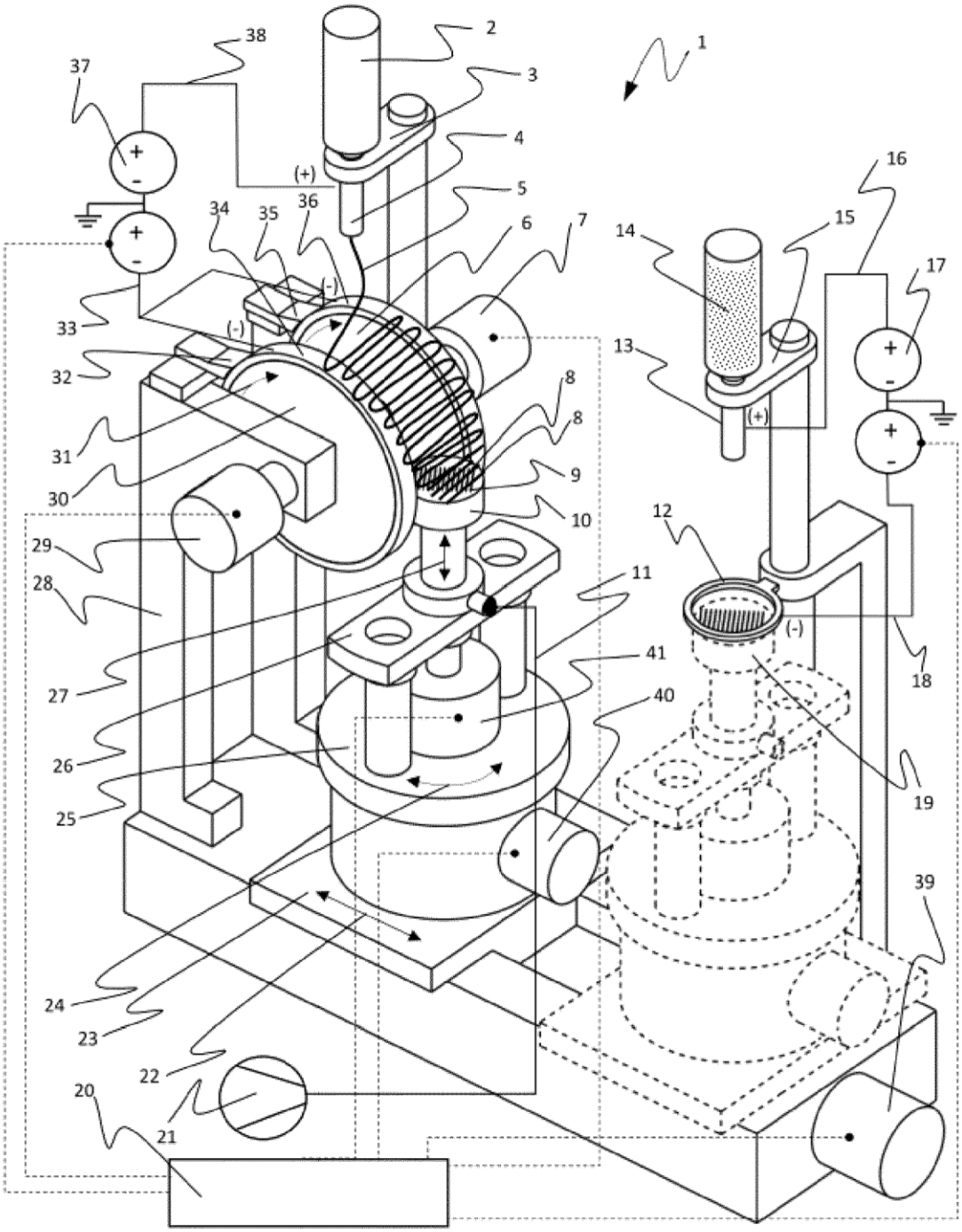
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Fig. 2

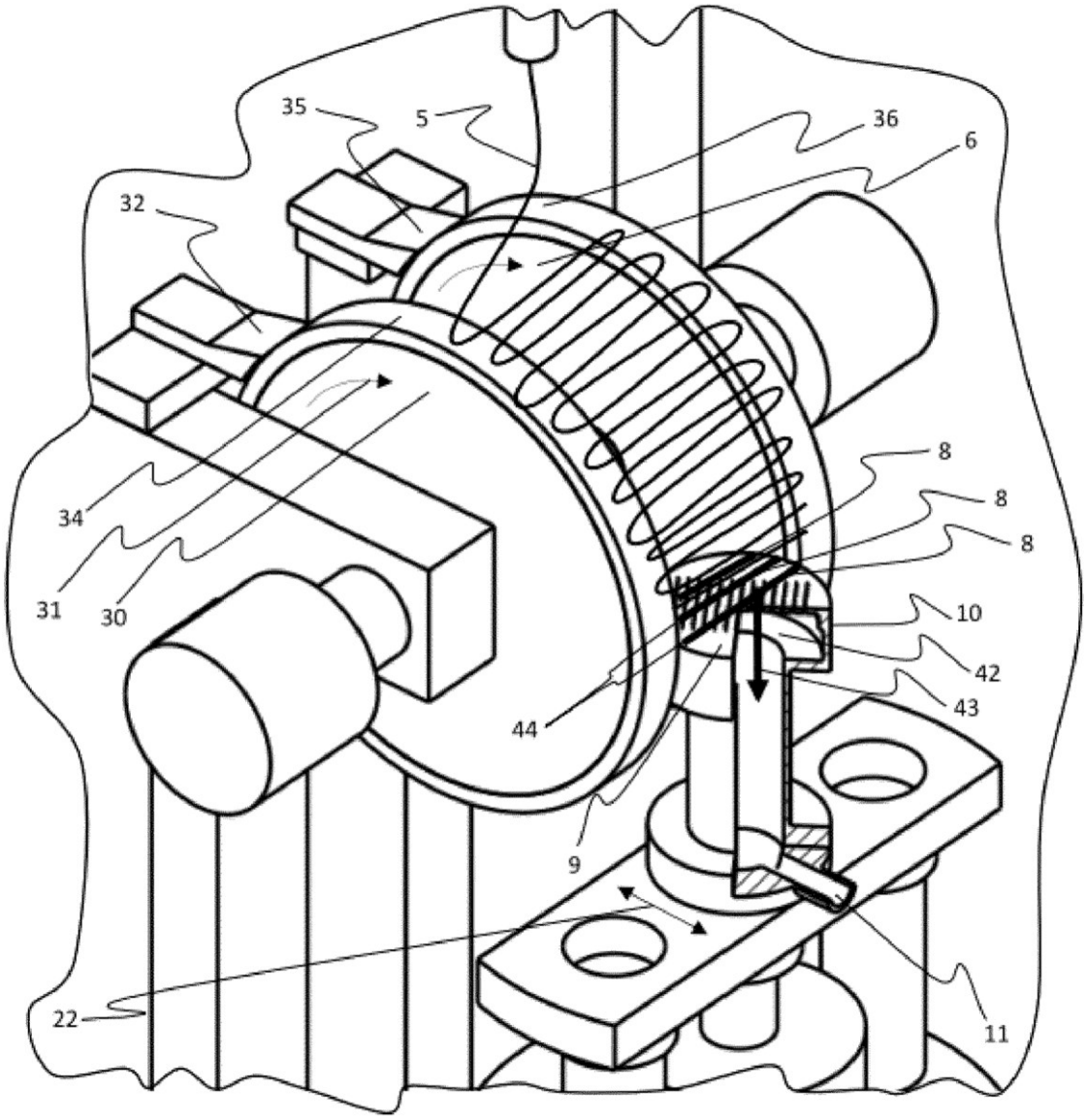

Fig. 3

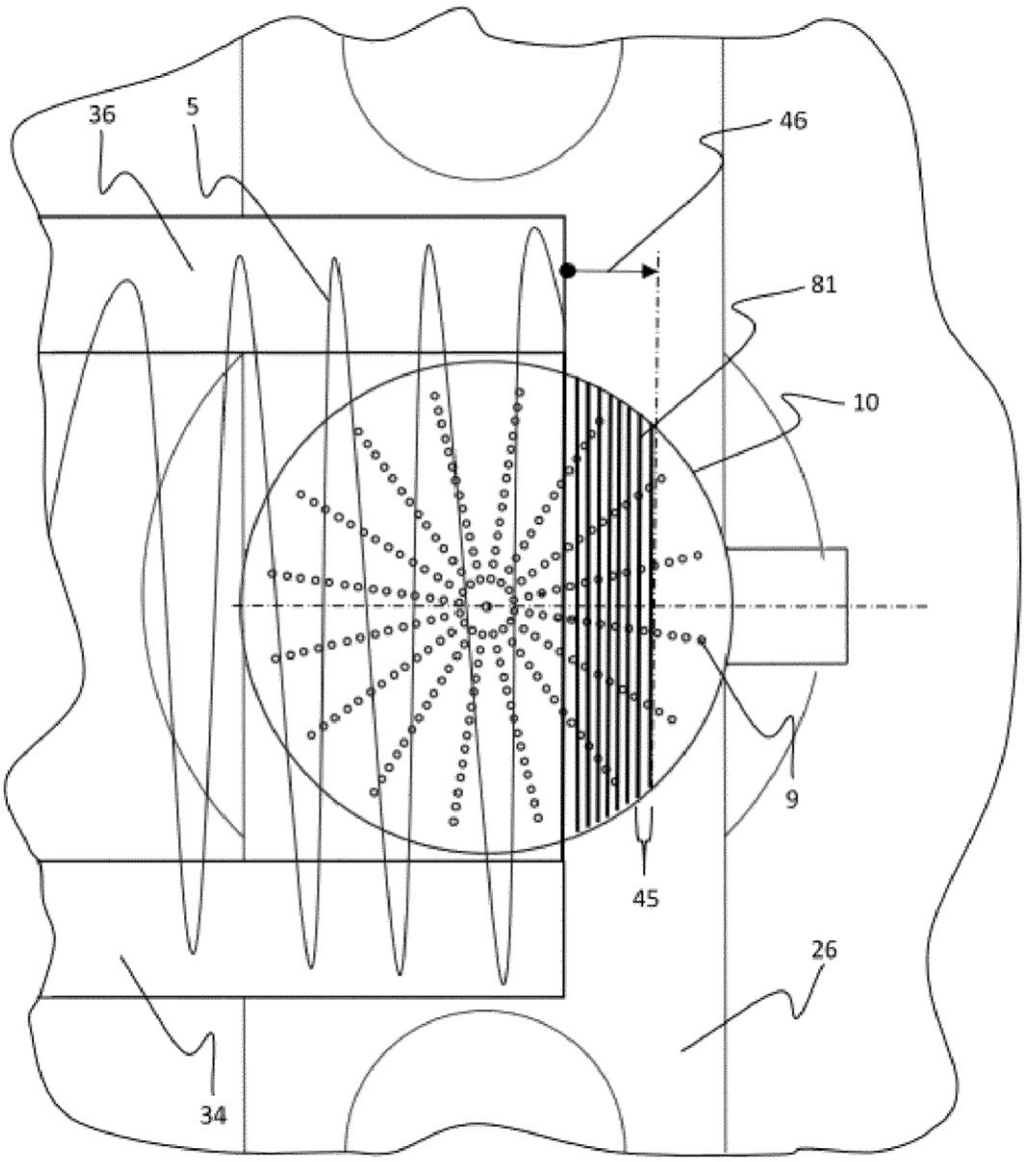

Fig. 4

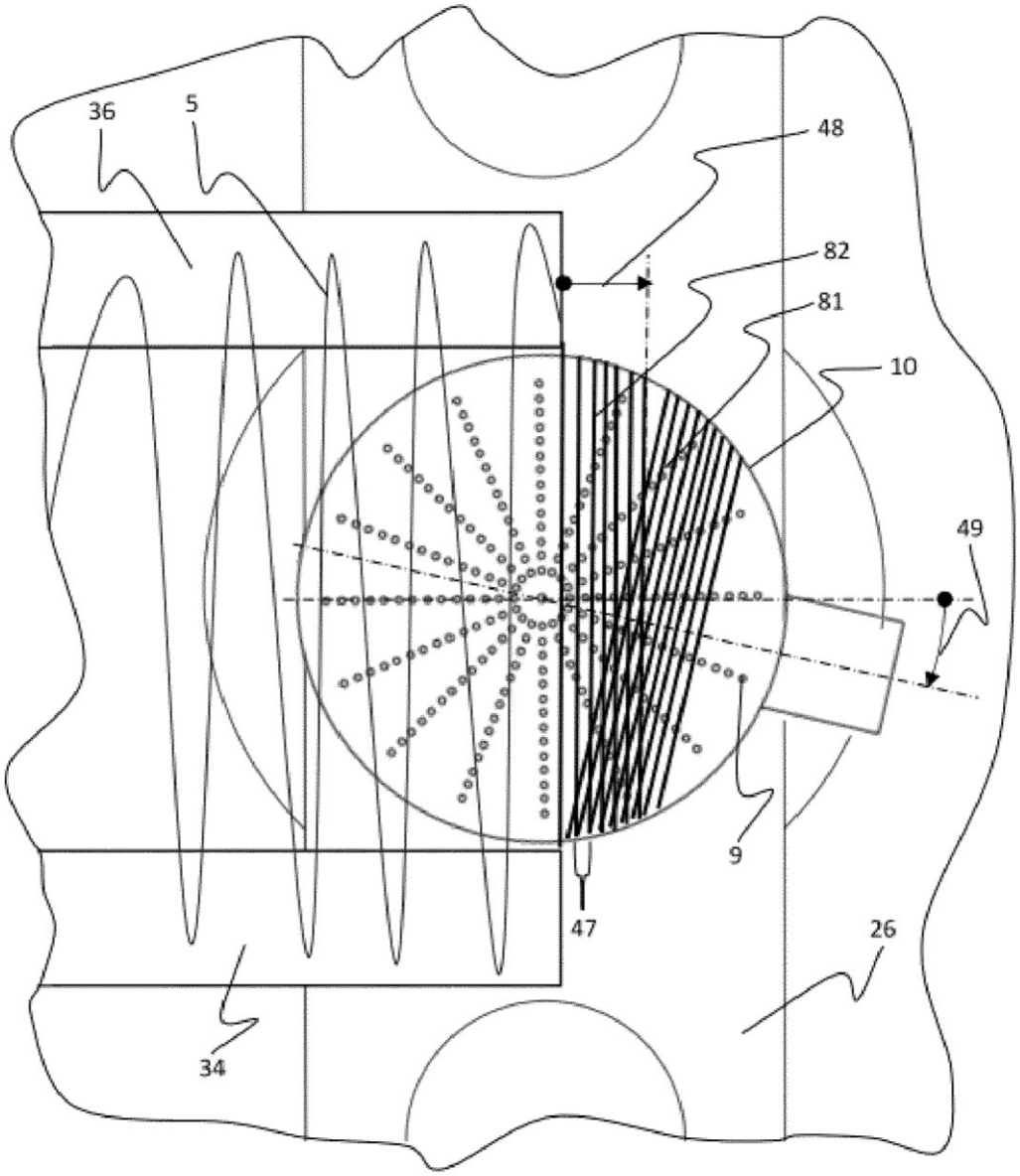

Fig. 5

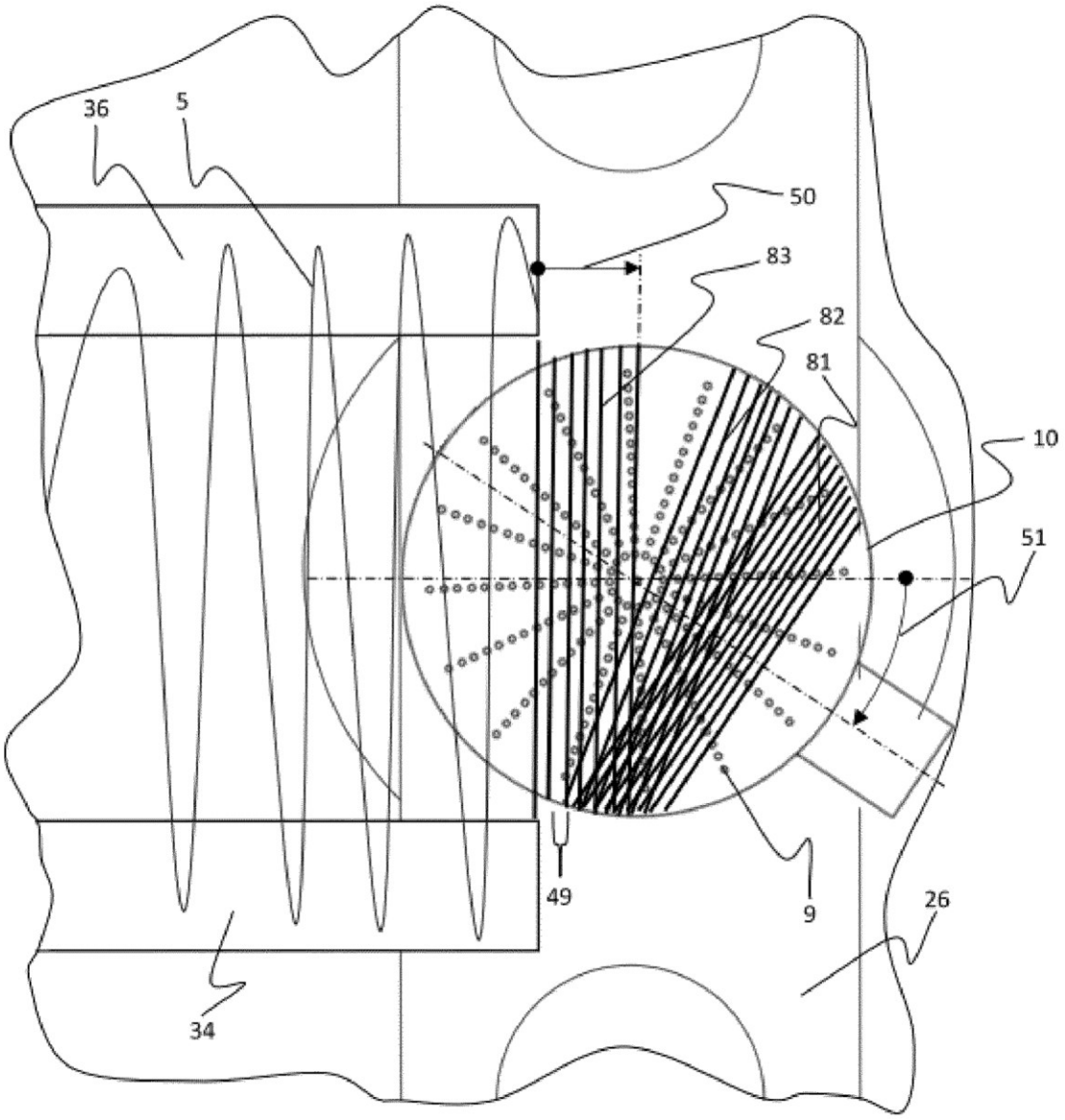

Fig. 6

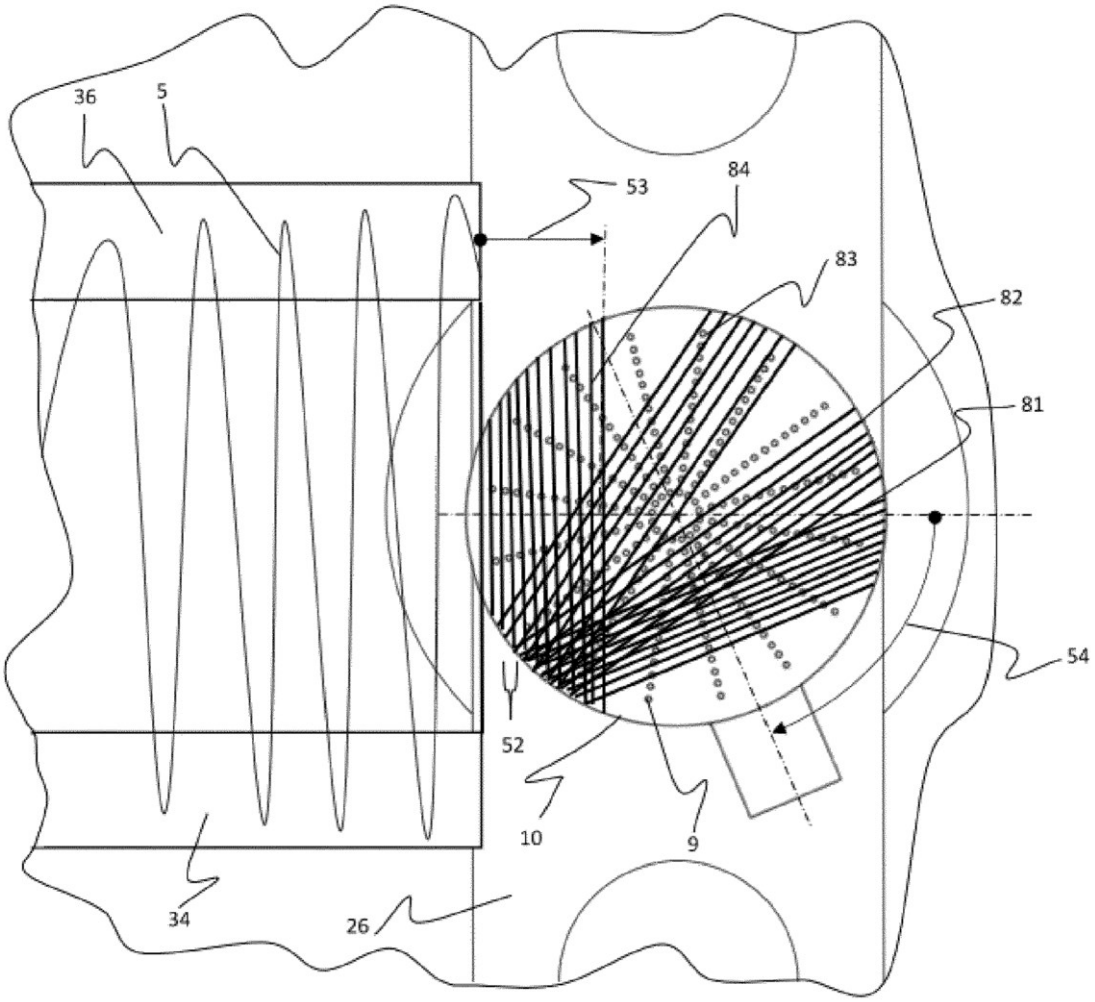

Fig. 7

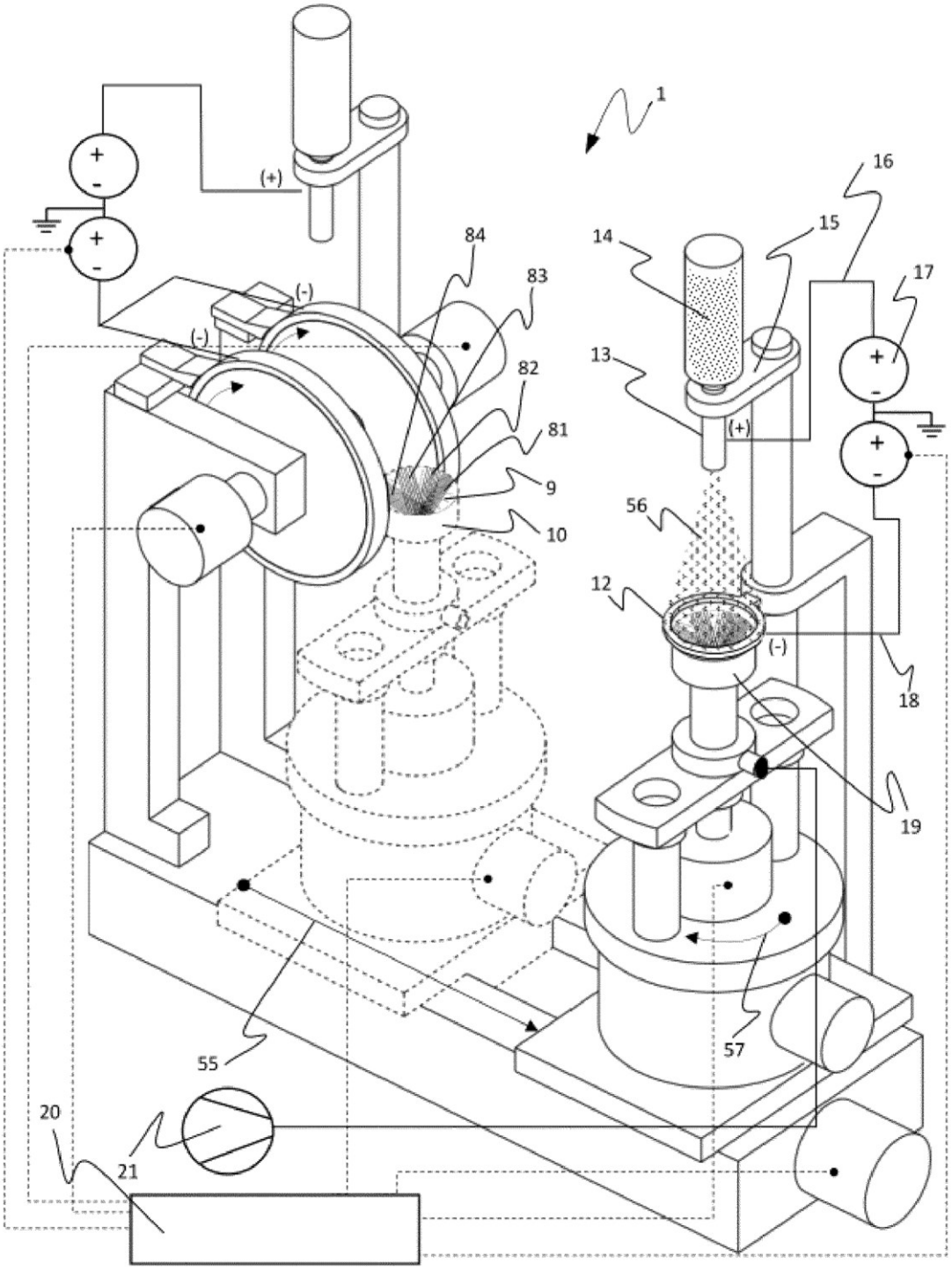

Fig. 8

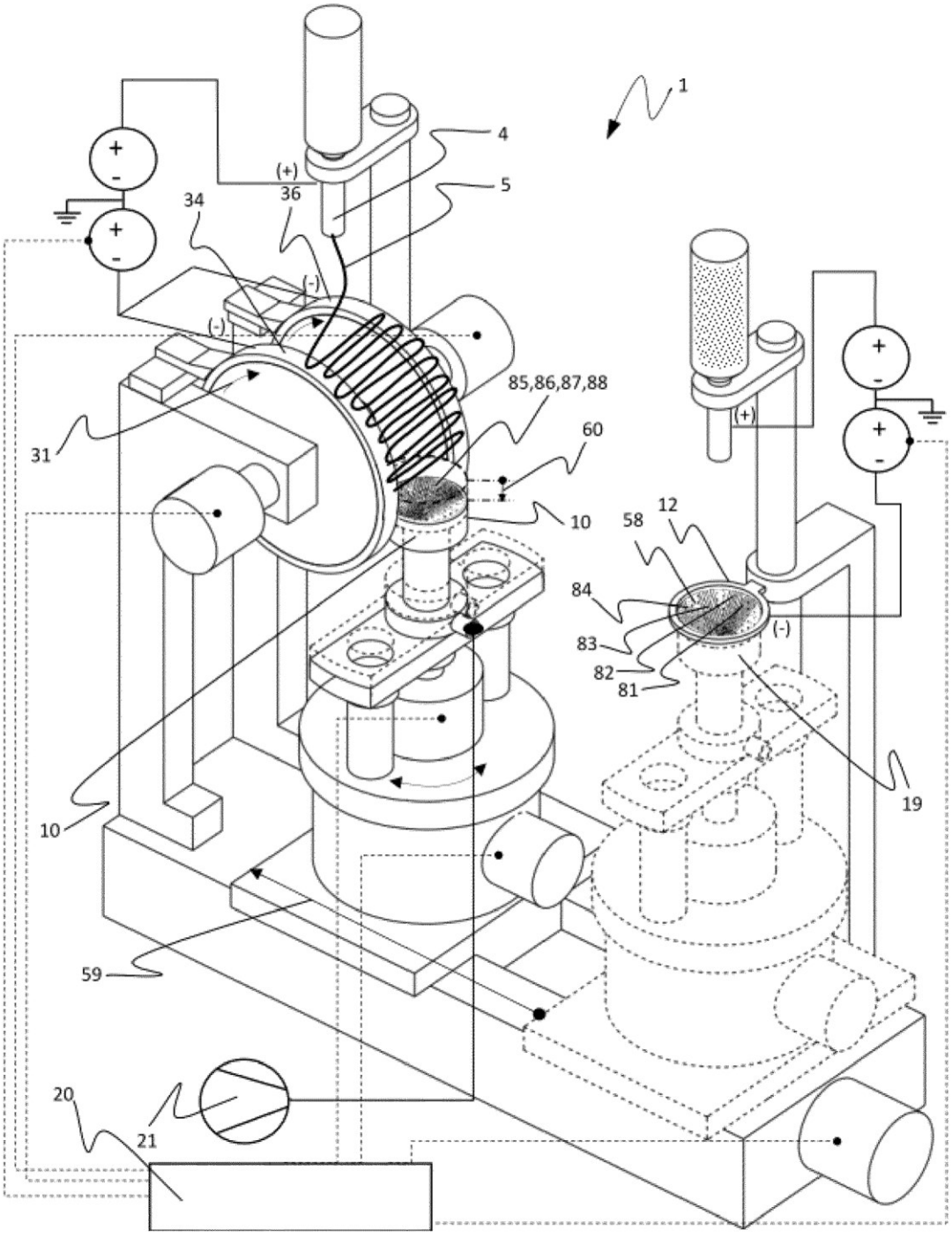

Fig. 9

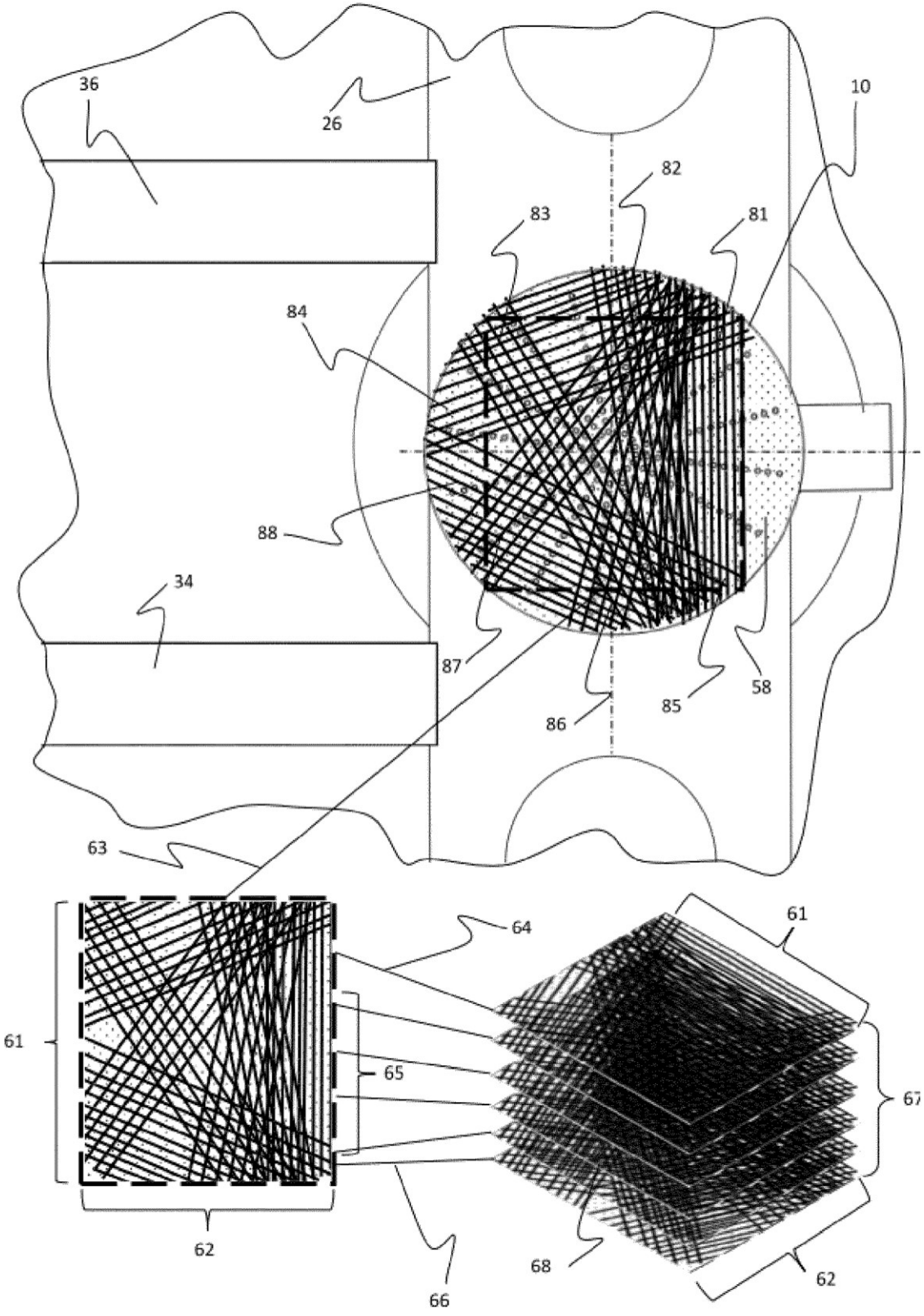

Fig. 10