

METHOD AND APPARATUS FOR MAKING THREE-DIMENSIONAL BIOMIMETIC SCAFFOLD WITH GRADUAL COILED COMPOSITE STRUCTURE

TECHNICAL FIELD

The present invention relates to a three-dimensional biomimetic scaffold technology, and more particularly, to a method and an apparatus for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure.

BACKGROUND

The three-dimensional biomimetic scaffold technology has important applications in three-dimensional cell cultures. The three-dimensional cell culture technology is directed to a technology in which a carrier with a three-dimensional structure is co-cultured with different kinds of cells in vitro, so that the cells can migrate and grow in the three-dimensional space structure of the carrier to form a three-dimensional cell-carrier complex. For an ordinary cell culture, cells gradually lose their original traits due to their proliferation in a changing environment in vitro, which is generally inconsistent with the environment in vivo. Animal experiments are performed completely in vivo, but due to the restriction of various internal factors in vivo and the interaction between the internal and external environments, the animal experiments become complicated. As a result, it is difficult to study a single process, nor an intermediate process. The three-dimensional cell culture technology is a technology between the single-layer cell culture and the animal experiment, which not only maximizes the simulation of the internal environment, but also shows the advantages of the intuitiveness and controllability of cell culture.

In order to successfully achieve a real sense of the three-dimensional biological culture, it is required to have a three-dimensional biomimetic scaffold with some important features. One is that the scaffold must have an interconnected porous structure to facilitate the ingrowth of cells and the transport of materials. The ingrowth of cells plays a key role in the formation of

new tissue. In addition, nutrients and oxygen are required to be transported into the interior of the scaffold, and metabolites and degradation products of the scaffold materials are also required to be efficiently excreted. The second is that the scaffold needs to have the mechanical properties that match a specific application. The mechanical properties of the scaffolds corresponding to different tissues are generally not the same, and in this case the matching of mechanical properties should be considered. The third is that the scaffold material needs to have good biocompatibility and degradability so as to avoid causing an immune reaction.

Therefore, what kind of process and apparatus can be used to obtain a three-dimensional cell culture scaffold or tissue engineering scaffold with a precise structure and a little batch difference has become an urgent problem to be solved. In addition, how to control the pore size and mechanical properties of the scaffold, and how to control the interaction between the scaffold and the biological system, i.e., how to achieve the invasive growth of the cells, etc., are problems required to be studied before the technology is applied to the really useful biomedicine.

SUMMARY

In view of the above, it is necessary to provide a method and an apparatus for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure aiming at the limitations and disadvantages of the three-dimensional biomimetic scaffold technology in the prior art. The method and the apparatus can utilize the principle of electrospinning to print a three-dimensional biomimetic scaffold with a gradual coiled composite structure, by adjusting the control parameters during printing process. The three-dimensional biomimetic scaffold with the gradual coiled composite structure made according to the method and the apparatus can provide a new choice for the three-dimensional cell culture technology and the tissue engineering scaffold. The three-dimensional biomimetic scaffold has a controllable multi-size

structure, porosity, and fiber diameter. The internal structure of the three-dimensional biomimetic scaffold has the characteristics of the gradual coiled composite structure, so it is more favorable for cell adsorption after cell inoculating, and for differentiation and proliferation during cell culture. The three-dimensional biomimetic scaffold can be for example, applied to three-dimensional cell culture carrier in vitro, and drug screening.

According to one aspect of the present invention, a method for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure is provided. The three-dimensional biomimetic scaffold includes a fiber unit with a plurality of layers of coiled structures. The method includes the following steps:

- determining a micro-appearance of the coiled structure of the three-dimensional biomimetic scaffold by setting or adjusting a structural parameter;

- preparing the coiled structure having the determined micro-appearance by electrohydrodynamic jet printing, based on the determined micro-appearance;

- selecting the number of the layers of the coiled structures in the fiber unit of the three-dimensional biomimetic scaffold, to determine a structural gradient characteristic of the fiber unit; and

- forming the coiled structures in the fiber unit layer by layer, based on the determined structure gradient characteristic of the fiber unit, and reducing coiled degrees of the layers of the coiled structures layer by layer, so that the fiber unit has a structure starting from a coiled shape at the bottom to a linear shape at the top.

In one embodiment, the micro-appearance includes a sine-shaped shape, an adjacent circular shape, and a separated circular shape.

In one embodiment, determining the micro-appearance of the coiled structure includes determining a micro-appearance of the coiled structure of the lowest layer in the plurality of layers of the coiled structures in the fiber unit of the three-dimensional biomimetic scaffold.

In one embodiment, the method further includes preparing a polycaprolactone solution for

forming the three-dimensional biomimetic scaffold.

In one embodiment, the method further includes generating a plurality of fiber units with different sizes.

The method for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure according to the present invention can manufacture a three-dimensional biomimetic scaffold with a gradual coiled composite structure and a multi-sized fiber unit (small to a few microns in size, and up to tens of microns in size), by accurately controlling the structural parameters in a porous tissue engineering scaffold. The polycaprolactone (PCL) material used for manufacturing has good biocompatibility and degradability, which can meet the performance requirements for the three-dimensional biomimetic culture scaffold and the tissue engineering scaffold.

According to another aspect of the present invention, an apparatus for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure is provided. The apparatus includes: a material supply unit including a storing container, an ejection nozzle and a pumping device, the pumping device being adapted to eject material stored in the storing container through the ejection nozzle; a high voltage power source adapted to generate a high voltage electrostatic field, the material ejected from the ejection nozzle forming material filaments under action of the high voltage electrostatic field, and the material filaments being adapted to form the coiled structure; a triaxial motion platform including a plurality of first linear motors and a stage, the plurality of first linear motors being adapted to drive the stage to move in a three-dimensional space; a base plate placed on the stage and below the ejection nozzle, the base plate being adapted to collect the material filaments; a constant temperature and humidity chamber, the material supply unit, the triaxial motion platform and the base plate being placed in the constant temperature and humidity chamber, and the constant temperature and humidity chamber being adapted to provide a constant temperature and humidity environment; a controller configured to receive an user input to

control operations of the pumping device, the high voltage power source and the triaxial motion platform, and further configured to set a control parameter prior to making the three-dimensional biomimetic scaffold, and to adjust the control parameter during making the three-dimensional biomimetic scaffold.

Preferably, adjusting the control parameter includes: changing a moving speed of the stage; changing a voltage value of the high voltage power source; changing a supply rate of the material supply unit; and changing a distance between the ejection nozzle and the base plate.

In one embodiment, the base plate is a polished silicon crystal base plate.

In one embodiment, the material is polycaprolactone solution.

In one embodiment, the apparatus further includes a second linear motor, the ejection nozzle is fixed to the second linear motor, and the second linear motor is adapted to be controlled by the controller to drive the ejection nozzle to move linearly in a vertical direction.

The three-dimensional biomimetic scaffold with the gradual coiled composite structure made by above method and apparatus according to the present invention is of benefit to adsorption of cells with different sizes during inoculation printing, and is also of benefit to proliferation of the cells on the biomimetic scaffold during subsequent growth. Moreover, the three-dimensional biomimetic scaffold with the gradual coiled composite structure has a porosity (for example, between 60% and 70%) suitable for cell growth, a relative large contact area, and a good pore connectivity, which is benefit to secretion and formation of extracellular matrix, as well as normal perfusion of culture solution, so that a good environment for the subsequent growth, differentiation and appreciation of the cells can be created. In addition, the material (e.g., polycaprolactone) used to make the three-dimensional biomimetic scaffold has good biocompatibility, which will not interfere with the body's immunologic mechanism and will not cause an immunological rejection.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a flow diagram illustrating a method for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure according to one embodiment of the present invention.

FIG. 2 shows types of micro-appearances of a base layer of the gradual coiled composite structure and corresponding images of the micro-appearance under an optical microscope.

FIG. 3 is a structural schematic diagram illustrating an apparatus for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure according to one embodiment of the present invention.

FIGS. 4A-4B are a top view and a side view of a three-dimensional biomimetic scaffold with a gradual coiled composite structure made according to one embodiment of the present invention under a scanning electron microscope respectively.

FIG. 5 shows images of fluorescent staining adherent cells cultured by a linear three-dimensional biomimetic scaffold and a three-dimensional biomimetic scaffold with a gradual coiled composite structure respectively for the first and the third day, under a confocal microscope.

DETAILED DESCRIPTION

Reference will now be made to the drawings to describe, in detail, embodiments of the present disclosure, so that the above objects, features and advantages of the present disclosure will be more apparent and understandable. In the following description, numerous specific details are set forth in order to provide a thorough understanding of the present invention. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the invention.

FIG. 1 is a flow diagram illustrating a method for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure according to one embodiment of the

present invention. The three-dimensional biomimetic scaffold includes a fiber unit with a plurality of layers of coiled structures. The structure of the fiber unit is a gradual coiled composite structure. That is, the bottom layer of the fiber unit is a complete coiled structure, and the top layer of the fiber unit is a linear structure. For a plurality of intermediate layers of coiled structures from the bottom layer to the top layer, their coiled degrees are reduces layer by layer. In this embodiment, the method for making the three-dimensional biomimetic scaffold with the gradual coiled composite structure includes the following steps.

S101: preparing a material for forming a three-dimensional biomimetic scaffold. The material is preferably polycaprolactone (PCL) solution. For example, the preparation process of the PCL solution may include: mixing a certain amount of PCL particles with a certain volume of glacial acetic acid; performing an ultrasonic bath on the mixed solution for 1 to 2 hours; stirring the mixed solution evenly; performing an ultrasonic bath on the mixed solution for 1 to 2 hours again; and obtaining a evenly mixed PCL solution with a concentration of 60wt% to 80wt%. PCL has good biocompatibility and degradability, which can meet the performance requirements for the three-dimensional biomimetic culture scaffold and the tissue engineering scaffold.

S102: determining a micro-appearance of the coiled structure of the three-dimensional biomimetic scaffold by setting or adjusting a structural parameter. As shown in FIG. 2, the micro-appearance includes a sine-shaped shape, an adjacent circular shape, and a separated circular shape. It should be understood by those skilled in the art that the micro-appearance of the coiled structure is not limited to those shown in FIG. 2, and any other suitable micro-appearance can be adopted according to actual needs. In this embodiment, determining the micro-appearance of the coiled structure includes determining the micro-appearance of the lowest layer of the coiled structure in the plurality of layers of the coiled structures in the fiber unit. The micro-appearances of the subsequent layers of the coiled structure can change gradually based on the micro-appearance of the lowest layer of

the coiled structure, i.e., the coiled degrees of the layers of the coiled structures decrease gradually to tend to be linear.

S103: preparing the coiled structure having the determined micro-appearance by electrohydrodynamic jet printing, based on the micro-appearance determined in the step S102. The coiled structure is a single layer structure in the fiber unit of the three-dimensional biomimetic scaffold. The electrohydrodynamic jet printing can be implemented using any electrohydrodynamic jet printing technology known or to be developed. Hereinafter, the method of the electrohydrodynamic jet printing in this embodiment will be specifically described in combination with the apparatus for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure according to the present invention, and is not described here.

S104: selecting the number of layers of the coiled structures in the fiber unit of the three-dimensional biomimetic scaffold, thereby determining a structural gradient characteristic of the fiber unit. The fiber unit includes a plurality of layers of coiled structures which accumulate one by one, and the coiled degrees of these layers of coiled structures decrease gradually from the bottom layer to the top layer until the top layer becoming linear. Therefore, as long as the number of layers of the coiled structures in the fiber unit is selected, the structural gradient characteristic of the fiber unit can be determined.

S105: forming the coiled structures in the fiber unit layer by layer, based on the determined structure gradient characteristic of the fiber unit, and reducing coiled degrees of the layers of the coiled structures gradually, so that the fiber unit has a structure starting from a coiled shape at the bottom to a linear shape at the top. In order to generate a fiber unit with a gradual coiled composite structure, a bottom layer of the coiled structure conforming to the micro-appearance determined in the step S102 is firstly generated, and then layers of the coiled structures with decreased coiled degrees are accumulated from the bottom layer of the coiled structure layer by layer, until a layer of the coiled structure approaches linear, and a

linear structure is finally generated on the top layer, so that the gradual change in the structure of the fiber unit from a coiled shape to a linear shape can be realized.

S106: generating a plurality of fiber units with different sizes. A plurality of fiber units with different sizes can be generated according to needs, to allow the fabricated three-dimensional biomimetic scaffold to have a fiber structure with various sizes (e.g., small to a few microns in size, and up to tens of microns in size).

An apparatus for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure that implements the above method is described below with reference to FIG. 3. The apparatus works with the principle of electrospinning and includes a material supply unit 1, a high voltage power source 2, a triaxial motion platform 3, a base plate 4, a constant temperature and humidity chamber 5 and a controller 6.

The material supply unit 1 includes a storing container 101, an ejection nozzle 102, and a pumping device 103. The pumping device 103 is adapted to eject the material stored in the storing container through the ejection nozzle. The material supply unit 1 can provide a stable material supply for electrohydrodynamic jet printing. In this embodiment, evenly mixed PCL solution is stored in the storing container 101. The ejection nozzle 102 is detachably fixed to a linear motor (not shown) that can move along the Z-axis and the ejection nozzle 102 is perpendicular to the base plate 4 located below it.

The positive electrode of the high voltage power source 2 is connected to the stage 301 of the triaxial motion platform 3, and the negative electrode is connected to the ejection nozzle 102. Preferably, both the stage 301 and the ejection nozzle 102 are made of metal. In this embodiment, the high voltage power source 2 is a DC high voltage power source. During operation of the apparatus, the high voltage power source 2 is suitable for generating a high voltage electrostatic field between the stage 301 and the ejection nozzle 102. The material ejected from the ejection nozzle 102 forms material filaments under action of a high voltage electrostatic field, and the material filaments are suitable for forming a coiled structure.

The triaxial motion platform 3 includes a plurality of linear motors (not shown) and a stage 301. Typically, it is provided with three linear motors which drive the movement of the stage 301 in three-dimensional space along the X-axis, Y-axis, and Z-axis, respectively. The base plate 4 is detachably fixed to the stage 301 and below the ejection nozzle 102, and the base plate 4 is adapted to collect the material filaments ejected from the ejection nozzle 10. In this embodiment, the base plate 4 is a polished silicon crystal substrate. It should be understood by those skilled in the art that the base plate can be made of any other suitable material.

The material supply unit 1, the triaxial motion platform 3 and the base plate 4 are placed in a constant temperature and humidity chamber 5 which is suitable for providing a constant temperature and humidity environment so as to ensure the constant temperature and humidity during the entire working process.

The controller 6 is configured to receive an user input to control operations of the pumping device 103, the high voltage power source 2 and the triaxial motion platform 3, for example, to control a moving position and a moving speed of the stage 301, a distance between an opening of the ejection nozzle 102 and a top surface of the base plate 4, an output voltage of the high voltage power source 2, a supply rate of the material supply unit 1 and the like. The controller 6 is also configured to set a control parameter prior to making the three-dimensional biomimetic scaffold and adjust the control parameter during making the three-dimensional biomimetic scaffold. Specifically, for example, the moving path and the moving speed of the stage 301 of the triaxial motion platform 3 are set prior to making the three-dimensional biomimetic scaffold, and the moving speed may generally be set to 50 mm/s to 300 mm/s. The distance between the opening of the ejection nozzle 102 and the top surface of the base plate 4 is set to, for example, a certain value in the range of 1.5 mm to 5 mm. The output voltage of the high voltage power source 2 is set to, for example, a certain value in the range of 2 kV to 4 kV. The supply rate of the material supply unit 1, i.e., the supply rate of the PCL solution, is set to, for example, a certain value in the range of 0.1 $\mu\text{l}/\text{min}$ to 10

$\mu\text{l}/\text{min}$. After the above control parameters are set, the high voltage power source 2 can be enabled to generate a high voltage electrostatic field between the stage 301 and the ejection nozzle 102. The pumping device 103 is driven to allow the PCL solution to be ejected from the opening of the ejection nozzle 102. The triaxial motion platform 3 is activated to allow the stage 301 to moves in a predetermined motion path, and the base plate 4 on the stage 301 receives the material filaments formed from the PCL solution in the high voltage electrostatic field, thereby forming a fiber unit.

During the process of generating the fiber unit, the controller 6 may adjust the control parameters. For example, the moving speed of the stage 301 may be adjusted, which allows the ejected material filament gradually to form a linear fiber during the movement of the stage after the speed change. The output voltage of the high-voltage power supply 2 may be adjusted, which allows to change the field strength of the high voltage electrostatic field. For example, when the generation of fiber units is approaching the end, a relative large voltage is output, so that the formed material filament is pulled downward by a larger electric field force and tends to form linear fiber. The supply rate of the pumping device 103 may be adjusted, which allows the pressure on the PCL solution in the storing container 101 to change after the supply rate is changed, so that the extrusion speed of the PCL solution is changed, and after the supply rate is increased, the pressure on the PCL solution is increased, and the material filaments tend to form linear fibers under this varying pressure. The distance between the opening of the ejection nozzle 102 and the top surface of the base plate 4 is adjusted, and this distance affects the field strength of the electrostatic field and the time required for the material filaments from the nozzle to the base plate 4, after the distance is reduced, the material filaments tend to form linear fibers.

According to the above method and apparatus of the present invention, a three-dimensional biomimetic scaffold with a gradual coiled composite structure can be manufactured by accurately controlling and adjusting a plurality of control parameters in the

preparation process of the three-dimensional biomimetic scaffold. FIGS. 4A-4B are a top view and a side view of a three-dimensional biomimetic scaffold with a gradual coiled composite structure under a scanning electron microscope respectively. The three-dimensional biomimetic scaffold with the gradual coiled composite structure is of benefit to the adsorption of cells with different sizes during inoculation printing, and is of benefit to the proliferation of cells on the biomimetic scaffold during subsequent growth. Moreover, the three-dimensional biomimetic scaffold with a gradual coiled composite structure has a porosity (for example, a porosity of 60%-70%) suitable for cell growth, a relative large contact area, and a good pore connectivity, which is benefit to the secretion and the formation of extracellular matrix, as well as the normal perfusion of the culture solution, so that a good environment for the subsequent growth, differentiation and appreciation of cells can be created.

FIG. 5 shows images of fluorescent staining adherent cells cultured by a linear three-dimensional biomimetic scaffold and a three-dimensional biomimetic scaffold with a gradual coiled composite structure for the first and the third day, under a confocal microscope. Referring to the confocal microscope images in FIG. 5, it can be seen that the effects of the adsorption and proliferation of cells on a three-dimensional biomimetic scaffold with a gradual coiled composite structure is better than that on a pure linear three-dimensional biomimetic scaffold under the same culture cycle. An absorbance analysis is performed for the cells on the two different scaffolds after staining MTS with a microplate reader. On the first day, the absorbance of the cells on the linear three-dimensional biomimetic scaffold is close to that on the three-dimensional biomimetic scaffold with the gradual coiled composite structure. The absorbance of cells on the linear three-dimensional biomimetic scaffold after staining is approximately 0.35, which is slightly lower than the absorbance of cells on the three-dimensional biomimetic scaffold with the gradual coiled structure, which is 0.40. However, on the third day, the absorbance of the cells on the linear three-dimensional biomimetic scaffold after culture and staining is approximately 0.43, while the absorbance of

cells on the three-dimensional biomimetic scaffold with the gradient coiled structure after staining is approximately 1.0. That means that the gradual coiled composite structure is benefit to the proliferation of the cells on the scaffold.

The foregoing examples are merely specific embodiments of the present invention, which are described in detail, but they are not intended to limit the protection scope of the present invention. It should be noted that any variation or replacement readily figured out by persons skilled in the art within the technical scope disclosed in the present invention shall all fall within the protection scope of the present invention. Therefore, the scope of the present invention is defined by the appended claims.

What is claimed is:

1. A method for making a three-dimensional biomimetic scaffold with a gradual coiled composite structure, the three-dimensional biomimetic scaffold including a fiber unit with a plurality of layers of coiled structures, the method comprising:

determining a micro-appearance of the coiled structure of the three-dimensional biomimetic scaffold by setting or adjusting a structural parameter;

preparing the coiled structure having the determined micro-appearance by electrohydrodynamic jet printing, based on the determined micro-appearance;

selecting the number of the layers of the coiled structures in the fiber unit of the three-dimensional biomimetic scaffold, to determine a structural gradient characteristic of the fiber unit; and

forming the coiled structures in the fiber unit layer by layer, based on the determined structure gradient characteristic of the fiber unit, and reducing coiled degrees of the layers of the coiled structures gradually, so that the fiber unit has a structure starting from a coiled shape at the bottom to a linear shape at the top.

2. The method of claim 1, wherein the micro-appearance comprises a sine-shaped shape, an adjacent circular shape, and a separated circular shape.

3. The method of claim 1, wherein determining the micro-appearance of the coiled structure comprises determining a micro-appearance of the coiled structure of the lowest layer in the plurality of layers of the coiled structures in the fiber unit of the three-dimensional biomimetic scaffold.

4. The method of claim 1, further comprising: preparing a polycaprolactone solution for forming the three-dimensional biomimetic scaffold.

5. The method of claim 1, further comprising: generating a plurality of fiber units with different sizes.

6. An apparatus for making a three-dimensional biomimetic scaffold with a gradual coiled

composite structure, the three-dimensional biomimetic scaffold comprising a fiber unit with a plurality of layers of coiled structures, the apparatus comprising:

a material supply unit comprising a storing container, an ejection nozzle and a pumping device, the pumping device being adapted to eject material stored in the storing container through the ejection nozzle;

a high voltage power source adapted to generate a high voltage electrostatic field, the material ejected from the ejection nozzle forming material filaments under action of the high voltage electrostatic field, and the material filaments being adapted to form the coiled structure;

a triaxial motion platform comprising a plurality of first linear motors and a stage, the plurality of first linear motors being adapted to drive the stage to move in a three-dimensional space;

a base plate placed on the stage and below the ejection nozzle, the base plate being adapted to collect the material filament;

a constant temperature and humidity chamber, the material supply unit, the triaxial motion platform and the base plate being placed in the constant temperature and humidity chamber, and the constant temperature and humidity chamber being adapted to provide a constant temperature and humidity environment;

a controller configured to receive an user input to control operations of the pumping device, the high voltage power source and the triaxial motion platform, and further configured to set a control parameter prior to making the three-dimensional biomimetic scaffold, and to adjust the control parameter during making the three-dimensional biomimetic scaffold, so that the apparatus for making the three-dimensional biomimetic scaffold with the gradual coiled composite structure is caused to:

determine a micro-appearance of the coiled structure of the three-dimensional biomimetic scaffold by setting or adjusting a structural parameter;

prepare the coiled structure having the determined micro-appearance by electrohydrodynamic jet printing, based on the determined micro-appearance;

select the number of the layers of the coiled structures in the fiber unit of the three-dimensional biomimetic scaffold, to determine a structural gradient characteristic of the fiber unit; and

form the coiled structures in the fiber unit layer by layer, based on the determined structure gradient characteristic of the fiber unit, and reduce coiled degrees of the layers of the coiled structures layer by layer, so that the fiber unit has a structure starting from a coiled shape at the bottom to a linear shape at the top.

7. The apparatus of claim 6, wherein adjusting the control parameter comprises: changing a moving speed of the stage; changing a voltage value of the high voltage power source; changing a supply rate of the material supply unit; and changing a distance between the ejection nozzle and the base plate.

8. The apparatus of claim 6, wherein the base plate is a polished silicon crystal base plate.

9. The apparatus of claim 6, wherein the material is polycaprolactone solution.

10. The apparatus of claim 6, further comprising: a second linear motor, wherein the ejection nozzle is fixed to the second linear motor, and the second linear motor is adapted to be controlled by the controller to drive the ejection nozzle to move linearly in a vertical direction.