

ELECTRO-FLUID JETTING DEVICE AND METHOD FOR PRINTING A THREE-DIMENSIONAL BIOLOGICAL SCAFFOLD FIELD

The present disclosure relates to the field of electrospinning micro-nanofiber engineering, and more particularly, to an electro-fluid jetting device for printing a three-dimensional biological scaffold with a microstructure and a method for printing the three-dimensional biological scaffold.

BACKGROUND

Tissue engineering takes principles of life sciences and engineering toward the development of functional alternatives to reconstruct, repair or improve a tissue function. Tissue engineering has been rapidly developed over the past two decades, especially in the last decade, has a potential to repair and reconstruct many human tissues and organs such as skin, bone and cartilage, liver, heart valves and blood vessels, bladder, pancreas, nerves, cornea, some other soft tissue and the like. Tissue engineering comprises three aspects: seeding cell, scaffold for the tissue engineering, and tissue construction (or growth factors). Among them, the porous scaffold plays an important role.

In order to achieve the purpose of a successful tissue repair, scaffolds for tissue engineering need some important features. Firstly, the scaffolds must have an interconnected porous structure to facilitate the cell ingrowth and material transmission. The cell ingrowth plays a key role in the formation of a new tissue; in addition nutrients and oxygen must be able to be transferred into the interior of the scaffolds, and metabolites and degradation products of the scaffold material can also

be effectively discharged. Secondly, the scaffold must have mechanical properties that match with specific applications. Different tissues correspond to different mechanical properties, and thus it is necessary to consider matching with mechanical properties. Thirdly, the scaffold material has a good biocompatibility and degradation, in order to avoid the induction of an immune response. In order to satisfy these important features of the scaffolds, many methods for printing scaffolds have been constantly developed.

As one of the methods for printing a three-dimensional biological scaffold, the key principle of the electrostatic spinning lies on applying a high voltage to a polymer solution so that the polymer solution forms a Taylor cone at a mouth of the nozzle. As the voltage continues to increase, the charged liquid forms a jet at the tip of the Taylor cone. The fibers are formed as the solvent evaporates during the drop of the jet, and then falls on the collector due to the electrostatic force. However, at present, the fiber ejected by the electrostatic spinning is difficult to form a scaffold in which the direction of the fiber arrangement is controllable and has disadvantages, such as low porosity, poor mechanical properties, low fiber fineness, and monotonicity of the structure. These shortcomings lead to the biological scaffold formed is not conducive to uniform distribution of cells and secretion and formation of extracellular matrix, and also cannot meet requirements for the adhesion and growth of cell with different sizes. Therefore, there is a need for improvement of the method for printing a three-dimensional scaffold using the electrostatic spinning, so as to print a three-dimensional biological scaffold which has a microstructure with various spiral structures.

SUMMARY

Based on this, in view of the above problems existing in the prior art, there is provided an electro-fluid jetting device and method for printing a three-dimensional biological scaffold facilitating the cell adhesion and growth by the same.

According to one aspect of the present disclosure, there is provided an electro-fluid jetting device for printing a three-dimensional biological scaffold comprising:

- a solution supply system including an spray needle ;
- a motion platform driven by a driving mechanism to move along the X, Y, Z axes;
- a motion control system which is connected to the driving mechanism and controls the movement of the driving mechanism;
- a carrier facing a nozzle of the spray needle and connected to the motion platform so as to move with the motion platform along the X and Y axes;
- a power source which is connected to and powers the driving mechanism and the solution supply system respectively.

In one embodiment, the spray needle is arranged along the Z-axis of the motion platform and controls the driving mechanism to move along the Z-axis of the motion platform by means of the motion control system so as to adjust the distance from the spray needle to the carrier.

In one embodiment, the solution supply system further includes an injector, a plastic hose and an injection pump, the injection pump, the injector, the plastic hose and the spray needle being sequentially connected with one another; a grounding electrode of the power source is connected to the spray needle and a positive pole of the power source is connected to the carrier.

In one embodiment, the electro-fluid jetting device further includes a temperature control device for regulating the temperature of an area where the motion platform is located, the temperature control device including a heating pipe and a temperature control switch electrically connected to the heating pipe.

According to another aspect of the present disclosure, there is provided

A method for printing a three-dimensional biological scaffold using the electro-fluid jetting device according to any one of claims 1 to 4, wherein the carrier of the electro-fluid jetting device is provided with a polished silicon crystal substrate, the method comprising:

- i) adding a polycaprolactone (PCL) solution mixed in glacial acetic acid into the solution supply system;
- ii) setting up a moving speed and a moving path of the motion platform during printing by the motion control system, adjusting a distance from the spray needle to the polished silicon crystal substrate, and setting up an injection rate of the solution;
- iii) turning on the power source and applying a voltage between the spray needle and the carrier.

In one embodiment, the PCL solution has a concentration of 70% by weight of PCL.

In one embodiment, the method for printing a three-dimensional biological scaffold further comprises setting up a temperature from 26°C to 30 °C at the area where the motion platform is located.

In one embodiment, a whipping jet is obtained between the spray needle and the carrier after the step of turning on the power source and applying a voltage between

the spray needle and the carrier.

In one embodiment, the voltage between the spray needle and the carrier is set as from 2 kV to 4 kV, the moving speed of the motion platform along the X and Y axes is set from 10 mm/s to 300 mm/s, respectively, the distance from the spray needle to the polished silicon crystal substrate is set as from 2.5 mm to 5 mm, and the injection rate of the solution is set as from 0.1 μ l/min to 10 μ l/min.

In one embodiment, the voltage between the spray needle and the carrier is set as 2.55 kV, the moving speed of the motion platform along the X and Y axes is set as 60 mm/s, the distance from the spray needle to the polished silicon crystal substrate is set as 3.5 mm, and the injection rate of the solution is set as 1 μ l/min.

In one embodiment, the voltage between the spray needle and the carrier is set as 2.55 kV, the moving speed of the motion platform along the X and Y axes is set as 100 mm/s, the distance from the spray needle to the polished silicon crystal substrate is set as 3.5 mm, and the injection rate of the solution is set as 1 μ l/min.

In one embodiment, the voltage between the spray needle and the carrier is set as 2.55 kV, the moving speed of the motion platform along the X and Y axes is set as 80 mm/s, the distance from the spray needle to the polished silicon crystal substrate is set as 3.5 mm, and the injection rate of the solution is set as 1 μ l/min.

In one embodiment, the voltage between the spray needle and the carrier is set as 3.5 kV, the moving speed of the motion platform along the X and Y axes is set as 60 mm/s, the distance from the spray needle to the polished silicon crystal substrate is set as 3.5 mm, and the injection rate of the solution is set as 6 μ l/min.

In comparison with prior art, the disclosure provides the following benefits:

(1) The electro-fluid jetting device of the present disclosure is capable of printing three-dimensional biological scaffolds with a microstructure with various spiral structures, which are in favor of the adhesion of cells with different sizes during inoculation, so that the cells are homogeneously distributed in the scaffolds. Under the same culture conditions and culture cycle, the adhesion and proliferation effects of the cells on the three-dimensional biological scaffolds microstructure with various spiral structures were significantly superior to those on the grid scaffolds.

(2) The electro-fluid jetting device of the present disclosure can produce a micro-scale fiber having a high resolution, a good guidance quality and a complicated structure.

(3) The electro-fluid jetting device of the present disclosure produces scaffolds for tissue engineering with a three-dimensional bionic structure by controlling the movement of the motion platform through the motion control system, and thus precisely controlling the spatial pattern and direction of the fiber.

(4) The three-dimensional scaffolds with a microstructure having various spiral structures printed by the electro-fluid jetting device of the present disclosure have a high porosity, a large contact area and a good pore connectivity, facilitate the secretion and formation of the extracellular matrix and the normal perfusion of the culture medium, and provide a good environment for the subsequent growth, differentiation and proliferation of cells.

(5) The material used in the three-dimensional biological scaffolds with a microstructure having various spiral structures of the present disclosure has a good biocompatibility and it neither interfere with the immune mechanism of the body nor

induce an immune rejection response.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a structural schematic diagram of an electro-fluid jetting device according to one embodiment of the present disclosure;

Figure 2 is a schematic diagram of the principle for printing a three-dimensional biological scaffold with a microstructure having various spiral structures using an electro-fluid jetting device according to one embodiment of the present disclosure;

Figure 3 is an optical image of a three-dimensional scaffold with a sinusoidal microstructure printed by the electro-fluid jetting device according to one embodiment of the present disclosure;

Figure 4 is an optical image of a biological scaffold of a multistage micrometer-scale structure with a microstructure having circles in a discrete form printed by the electro-fluid jetting device according to one embodiment of the present disclosure;

Figure 5 is an optical image of a biological scaffold of a multistage micrometer-scale structure with a microstructure having circles in an adjacent form printed by the electro-fluid jetting device according to one embodiment of the present disclosure;

Figure 6 is an optical image of a biological scaffold of a multistage micrometer-scale structure with a microstructure having circles in a staggered form printed by the electro-fluid jetting device according to one embodiment of the present disclosure;

Figure 7 is an optical image of a three-dimensional biological scaffold with a double layered sinusoidal microstructure printed by the electro-fluid jetting device according to one embodiment of the present disclosure;

Figure 8 is an optical image of a three-dimensional biological scaffold of a

multistage micrometer-scale structure with a double layered microstructure having circles in a discrete form printed by the electro-fluid jetting device according to one embodiment of the present disclosure;

Figures 9 and 10 are photographs of the adhesion and proliferation effects of the cells on a grid structural scaffold and the spiral structural scaffold printed by the electro-fluid jetting device according to the present disclosure under the same culture conditions and culture cycle, respectively.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, specific embodiments of the present disclosure will be described in detail with reference to the accompanying drawings. These embodiments, which are carried out under the technical solutions of the present disclosure, set out the detailed implementation and operation processes; however, the protection scope of the present disclosure is not limited to the following embodiments.

As shown in Fig. 1, an electro-fluid jetting device for printing a three-dimensional biological scaffold comprises:

- a solution supply system 1 including an spray needle 8 ;
- a motion platform 2 driven by a driving mechanism to move along the X, Y, Z axes;
- a motion control system 3 which is connected to the driving mechanism and controls the movement of the driving mechanism;
- a carrier 16 facing a nozzle of the spray needle and connected to the motion platform so as to move with the motion platform along the X and Y axes;
- a power source 4 which is connected to the driving mechanism and the solution

supply system respectively and applies a voltage between the carrier 16 and the spray needle 8 of the solution supply system to form an electrostatic field. In the present embodiment, the X, Y, and Z axes are perpendicular to one another.

Particularly, the driving mechanism may include a linear motor 13 for driving the motion platform 2 to move along the Y axis, a linear motor 14 for driving the motion platform 2 to move along the X axis, and a linear motor 15 for driving the motion platform 2 to move along the Z axis. The motion control system 3 controls the movement of the three linear motors, so that the motion platform moves along the X, Y, Z axis in three directions.

More particularly, the carrier 16 may be connected to the motion platform 2 and driven by the linear motor 14 and the linear motor 13 to move along the X and Y axes so that the carrier 16 moves on the plane formed by the X axis and the Y axis.

In the present embodiment, the power source 4 is a direct current high voltage power supply; its positive electrode is connected to the carrier 16 and its grounding electrode is connected to the spray needle 8 of the solution supply system 1, so that a solution supplied by the solution supply system 1 creates an electrical charge which forms an electrostatic field between the carrier 16 and the spray needle 8 to induce the formation of a Taylor cone. In an alternative embodiment, the positive electrode and the grounding electrode of the power supply 4 may be connected reversely.

In the present embodiment, the spray needle 8 is arranged along the Z-axis of the motion platform 2 and is opposed to the carrier 16, and the driving mechanism is controlled by the motion control system 3 so that the motion platform 2 moves along the Z-axis to adjust the distance from the spray needle 8 to the carrier 16. Particularly,

the spray needle 8 is a flat-end spray needle and can be removably fixed to the Z axis of the motion platform 2.

The spray needle 8 may be made of various suitable materials, for example, a stainless steel material, and it may have an inner diameter of 510 μ m.

In this embodiment, the carrier 16 is used for the installation of the polished silicon crystal substrate 12. The spray needle 8 is perpendicular to the polished silicon crystal substrate 12.

In this embodiment, the solution supply system 1 may further include an injector 6, a plastic hose 7 and an injection pump 9 which may provide a stable injection rate. The injection pump 9, the injector 6, the plastic hose 7 and the spray needle 8 are sequentially connected with one another.

In this embodiment, the electro-fluid jetting device may further include a temperature control device 5 for regulating the temperature of an area where the motion platform 2 is located.

Particularly, the temperature control device 5 includes a heating pipe 10 and a temperature control switch 11 capable of measuring and controlling the temperature. The heating pipe 10 is electrically connected to the temperature control switch 11. In an alternative embodiment, a plurality of heating pipes 10 may be evenly distributed over the area where the motion platform 2 is located.

The electric fluid jetting device of the present disclosure is configured to print a three-dimensional biological scaffold with a principle of near-electric field electrospinning, and its working principle is shown in Fig.2.

A method for printing a three-dimensional biological scaffold using the above-

mentioned electro-fluid jetting device of the present disclosure is described in detail as followings:

- i) adding a polycaprolactone (PCL) solution mixed in glacial acetic acid into the solution supply system;
- ii) setting up a moving speed and a moving path of the motion platform during printing by the motion control system, adjusting the distance from the spray needle to the polished silicon crystal substrate, and setting up an injection rate of the solution;
- iii) turning on the power source and applying a voltage between the spray needle and the carrier.

In this embodiment, the method for printing the three-dimensional biological scaffold can be as following: firstly, adding the polycaprolactone solution mixed in glacial acetic acid, for example, the polycaprolactone solution with a concentration of 70% by weight, into the injector; then, setting up the moving speed and the moving path of the motion platform by the motion control system, for example, the moving speed being set as from 10mm/s to 300mm/s; setting up the distance from the flat-end spray needle to the polished silicon crystal substrate from 2.5mm to 5mm, and setting up an injection rate of the solution from 0.1 μ l/min to 10 μ l/min. Meanwhile, a temperature can be set as from 26°C to 30°C at the area where the motion platform is located by the temperature control device .

The direct current high voltage power supply subsequently is turned on, and a voltage, for example, 2kV ~ 4kV, is applied between the flat-end spray needle and the carrier, thus forming an electrostatic field. A PCL jet is ejected from a Taylor cone at the tip of the flat-end spray needle, resulting in a continuous PCL fiber. The PCL

fiber forms a whipping jet, falls into the polished silicon crystal substrate fixed to the carrier and forms a whipping jet arranged in a spatial spiral conical distribution.

By adjusting the relation between the whipping jet and the carrier and precisely controlling the direction for distributing the PCL fiber on the silicon crystal substrate through moving the motion platform, the three - dimensional biological scaffolds of multistage micrometer-scale structures with a microstructure having various spiral structure can be printed.

When referring to the printed three - dimensional biological scaffold of a multistage micrometer-scale structure with a spiral microstructure, the microstructure means that the three-dimensional biological scaffold has a microscopic pattern; the multistage micrometer-scale structure means the three-dimensional biological scaffold under a microstructure has different pore sizes, fiber diameters and the microscopic patterns.

Several microstructures are as followings: one kind of the microstructure is one having a curve pattern which includes curve patterns that are irregularly arranged and regularly arranged. As shown in Fig.3, the typical curve pattern that is regularly arranged is a sinusoidal microstructure, in which the fiber is arranged as a sinusoid; another kind of the microstructure is one with a circle pattern. The main feature of the microstructure with a circle pattern is that the printed fiber can form a circle successively. According to the arrangement between the circles, the microstructures with a circle pattern are divided into: a microstructure having circles in a discrete form, as shown in Fig. 4, the circle fibers distancing from each other; and a microstructure having circles in an adjacent form, as shown in Fig. 5, the circular

fibers being closely arranged adjacent to each other; a microstructure having circles in a staggered form, as shown in Fig. 6 , circle fibers being in a staggered and overlapping arrangement.

The three-dimensional biological scaffolds may be formed by staggeredly stacking layers of PCL fibers, each of fibrous layers being consisted of a fibrous layer with a microstructure. For example, the three-dimensional biological scaffold with a double layered sinusoidal microstructure shown in Fig. 7 is consisted of two layers of fibrous layers having a sinusoidal microstructure, and the three-dimensional biological scaffold with a double layered microstructure having circles in a discrete form shown in Fig. 8 is consisted of two layers of fibrous layers having a microstructure having circles in a discrete form.

The electro-fluid jetting device of the present disclosure is configured to print three-dimensional biological scaffolds of multistage micrometer-scale structures with different microstructures by near-field electrospinning. Its working principle is shown in Fig.2.

The examples of printing three-dimensional biological scaffolds of multistage micrometer-scale structures with different microstructures by the above mentioned electro-fluid jetting device and its working principle are described as followings.

Example 1

The printing of a three-dimensional biological scaffold of a multistage micrometer-scale structure with a sinusoidal microstructure

i) Polycaprolactone (PCL) particles were mixed with glacial acetic acid to prepare a PCL solution having a concentration of 70% by weight, and then the PCL

solution was added into an injector;

ii) the distance D from the flat-end spray needle to the polished silicon crystal substrate was set as 3.5mm, the injection rate R of the solution was set as 1 μ l/min by the injection pump and the moving speeds of the motion platform along the X and Y-axes were set as 60mm/s;

iii) the voltage V between the spray needle and the carrier was set as 2.55kV.

The pattern of the three -dimensional biological scaffold printed by the above process is shown in Fig.3 and the pattern is characterized by its fiber with a sinusoidal microstructure.

Example 2

The printing of a three-dimensional biological scaffold of a multistage micrometer-scale structure with a microstructure having circles in a discrete form

i) Polycaprolactone (PCL) particles were mixed with glacial acetic acid to prepare a PCL solution having a concentration of 70% by weight, and then the PCL solution was added into an injector.

ii) the distance D from the flat-end spray needle to the polished silicon crystal substrate was set as 3.5mm, the injection rate R of the solution was set as 1 μ l/min by the injection pump and the moving speeds of the motion platform along the X and Y-axes were set as 100mm/s;

iii) the voltage V between the spray needle and the carrier was set as 2.55kV.

The pattern of the three -dimensional biological scaffold printed by the above process is shown in Fig. 4 and the pattern is characterized by its fiber with a microstructure having circles in a discrete form.

Example 3

The printing of a three-dimensional biological scaffold of a multistage micrometer-scale structure with a microstructure having circles in an adjacent form

i) Polycaprolactone (PCL) were mixed with glacial acetic acid to prepare a PCL solution having a concentration of 70% by weight, and then the PCL solution was added into an injector;

ii) the distance D from the flat-end spray needle to the polished silicon crystal substrate was set as 3.5mm, the injection rate R of the solution was set as 1 μ l/min by the injection pump and the moving speeds of the motion platform along the X and Y-axis were set as 80mm/s;

iii) the voltage V between the spray needle and the carrier was set as 2.55kV.

The pattern of the three -dimensional biological scaffold printed by the above process is shown in Fig. 5 and the pattern is characterized by its fiber with a microstructure having circles in an adjacent form.

Example 4

The printing of a three-dimensional biological scaffold of a multistage micrometer-scale structure with a microstructure having circles in a staggered form

i) Polycaprolactone (PCL) particles were mixing with glacial acetic acid to prepare a PCL solution having a concentration of 70% by weight, and then the PCL solution was added into an injector;

ii) the distance D from the flat-end spray needle to the polished silicon crystal substrate was set as 3.5mm, the injection rate R of the solution was set as 6 μ l/min by

the injection pump and the moving speeds of the motion platform along the X and Y-axis were set as 60mm/s;

iii) the voltage V between the spray needle and the carrier was set as 3.5kV.

The pattern of the three-dimensional biological scaffold printed in the above process is shown in Fig. 6 and the pattern is characterized by its fiber with a microstructure having circles in a staggered form.

Comparison experiments for cell culture were carried out on the three-dimensional linear biological scaffolds and biological scaffolds with a microstructure having spiral structures printed by the jetting device of the present disclosure. The results are shown in Fig. 9 and Fig. 10. Fig. 9 shows the photograph of cell culture using the existing grid structural scaffold. Fig. 10 shows the photograph of the cell culture at the same culture cycle using a three-dimensional biological scaffold with a microstructure having spiral structures. It can be seen from the photographs that the propagation speed of the cells on the three-dimensional biological scaffold with a microstructure having spiral structures is significantly faster than that on the linear grid scaffold.

The embodiments described above are merely illustrative of several embodiments of the present disclosure, which are more specific and detailed but should not be construed as limiting the scope of the present disclosure. It should be noted that various modifications and improvements can be made by those skilled in the art without departing from the concept of the present disclosure and are within the scope of the present disclosure. Accordingly, the scope of protection scope of the present disclosure should be determined by the appended claims.

Claims

1. An electro-fluid jetting device for printing a three-dimensional biological scaffold comprising:

a solution supply system including a spray needle ;

a motion platform driven by a driving mechanism to move along the X, Y, Z axes;

a motion control system which is connected to the driving mechanism and controls the movement of the driving mechanism;

a carrier facing a nozzle of the spray needle and connected to the motion platform so as to move with the motion platform along the X and Y axes;

a power source which is connected to and powers the driving mechanism and the solution supply system respectively.

2. The electro-fluid jetting device according to claim 1, wherein the spray needle is arranged along the Z-axis of the motion platform and controls the driving mechanism to move along the Z-axis of the motion platform by means of the motion control system so as to adjust the distance from the spray needle to the carrier.

3. The electro-fluid jetting device according to claim 1, wherein the solution supply system further includes an injector, a plastic hose and an injection pump, the injection pump, the injector, the plastic hose and the spray needle being sequentially connected with one another; a grounding electrode of the power source is connected to the spray needle and a positive pole of the power source is connected to the carrier.

4. The electro-fluid jetting device according to claim 1, wherein the electro-fluid jetting device further includes a temperature control device for regulating a

temperature of an area where the motion platform is located, the temperature control device including a heating pipe and a temperature control switch electrically connected to the heating pipe.

5. A method for printing a three-dimensional biological scaffold using the electro-fluid jetting device according to any one of claims 1 to 4, wherein the carrier of the electro-fluid jetting device is provided with a polished silicon crystal substrate, the method comprising:

- i) adding a polycaprolactone (PCL) solution mixed in glacial acetic acid into the solution supply system;
- ii) setting up a moving speed and a moving path of the motion platform during printing by the motion control system, adjusting a distance from the spray needle to the polished silicon crystal substrate, and setting up an injection rate of the solution;
- iii) turning on the power source and applying a voltage between the spray needle and the carrier.

6. The method for printing a three-dimensional biological scaffold according to claim 5, wherein the PCL solution has a concentration of 70% by weight of PCL.

7. The method for printing a three-dimensional biological scaffold according to claim 5, wherein the method for printing the three-dimensional biological scaffold further comprises setting up a temperature from 26°C to 30 °C at the area where the motion platform is located.

8. The method for printing a three-dimensional biological scaffold according to claim 5 or 6, wherein a whipping jet is obtained between the spray needle and the carrier after the step of turning on the power source and applying a voltage between

the spray needle and the carrier.

9. The method for printing a three-dimensional biological scaffold according to claim 8, wherein the voltage between the spray needle and the carrier is set as from 2 kV to 4 kV, the moving speed of the motion platform along the X and Y axes is set from 10 mm/s to 300 mm/s, respectively, the distance from the spray needle to the polished silicon crystal substrate is set as from 2.5 mm to 5 mm, and the injection rate of the solution is set as from 0.1 $\mu\text{l}/\text{min}$ to 10 $\mu\text{l}/\text{min}$.

10. The method for printing a three-dimensional biological scaffold according to claim 8, wherein the voltage between the spray needle and the carrier is set as 2.55 kV, the moving speed of the motion platform along the X and Y axes is set as 60 mm/s, the distance from the spray needle to the polished silicon crystal substrate is set as 3.5 mm, and the injection rate of the solution is set as 1 $\mu\text{l}/\text{min}$.

11. The method for printing a three-dimensional biological scaffold according to claim 8, wherein the voltage between the spray needle and the carrier is set as 2.55 kV, the moving speed of the motion platform along the X and Y axes is set as 100 mm/s, the distance from the spray needle to the polished silicon crystal substrate is set as 3.5 mm, and the injection rate of the solution is set as 1 $\mu\text{l}/\text{min}$.

12. The method for printing a three-dimensional biological scaffold according to claim 8, wherein the voltage between the spray needle and the carrier is set as 2.55 kV, the moving speed of the motion platform along the X and Y axes is set as 80 mm/s, the distance from the spray needle to the polished silicon crystal substrate is set as 3.5 mm, and the injection rate of the solution is set as 1 $\mu\text{l}/\text{min}$.

13. The method for printing a three-dimensional biological scaffold according to

claim 8, wherein the voltage between the spray needle and the carrier is set as 3.5 kV, the moving speed of the motion platform along the X and Y axes is set as 60 mm/s, the distance from the spray needle to the polished silicon crystal substrate is set as 3.5 mm, and the injection rate of the solution is set as 6 μ l/min.