printing also come from the system algorithm for organizing the printer. The printing plate corresponds to an individually created tool for printing and is preferably made of a polymer.

A pressure foil, preferably made of biodegradable or resorbable polymer, is applied above the pressure plate and has the same connections as the pressure plate. The printing foil is used for easy and non-destructive detachment of the tissue from the printing plate. Due to the biodegradability of the polymer, the printing film is degraded in the body in a short time (Figs. 2 -7).

In order to allow the printed tissue to mature without any problems in a commercially available incubator, the printing table with the printing plate (optionally only the printing plate) is transferred to an incubator vessel (Fig. 19), which is stored in the incubator. This incubator vessel consists in particular of a medium-filled tub with a lid to be placed on. A base is permanently installed on the bottom of the tub, on which a printer table base of the same construction is exchangeably attached. The supply and discharge medium hoses to the printer table base are routed through openings in the incubator vessel. The printer table including the printing plate, printing film and printed tissue is placed on the pins of the printer table base (without the silicone membrane) using magnetic force (Fig. 19).

Other embodiments:

In a preferred embodiment of the invention, the droplet size is between one femtoliter and 10 microliters. Advantageously, this droplet size allows the inventive task to be solved surprisingly well in a thiol-ene reaction. Thiol-modified molecules inside the drop do not crosslink under these conditions and are flushed out.

In another preferred embodiment, the capillary inks have thiol-modified molecules, the surrounding bioinks have allyl-modified molecules, and a photoinitiator to initiate the crosslinking reaction. This cross-linking reaction takes place within the bio-inks between the allyl-modified molecules. The thiol-modified molecules of the capillary inks cross-link only

selectively in the edge area with the allyl-modified molecules of the bio-inks.

In another preferred embodiment, a copper(I)-catalyzed 1,3-dipolar cycloaddition of azides and alkynes leads to a cross-linking reaction in the edge area of the capillary inks, with azide-modified and alkyne-modified molecules only meeting in the boundary area of the capillary ink droplets to form bioinks.

In a further preferred embodiment, the capillary ink contains epoxide group-modified molecules which can cause nucleophilic ring opening with OH groups or NH_2 groups of molecules from the bioinks or which undergo acid-catalyzed or base-catalyzed ring opening by components from the bioinks.

In another preferred embodiment, capillary inks and bioinks contain molecules that form self-assembling structures in the size range, for example via ions, aptamers or other structures that tend to self-assembling.

In a further preferred embodiment of the invention, it is provided that the cells contain a selective factor by which they are selectively crosslinked within a droplet by means of directed movement of the electromagnetic waves.

The new low-viscosity bioinks and capillary inks that can be crosslinked by electromagnetic waves are made up of a) the respective cell type (optionally with a factor that is selective for the cell type) and crosslinking molecules.). In addition, growth factors, signal molecules, particles for the development of an impulse conduction or other molecules can also be components of the ink.

A selectively acting energy source can trigger a cross-linking or polymerisation reaction from a droplet containing a mixture of different cells, in which only the intended cell type is hit. Preferably, the non-crosslinked cells can be removed. For example, a small droplet as small as 5 picoliters (about 20-200 cells) may consist of a mixture of vascular endothelial cells and myoblasts. In a preferred embodiment, the ring of a fine capillary is then formed from this

mixture using a nanometer-precise laser beam. By means of a specific input of energy from a laser 1, only the endothelial cells that are located along the inside of the ring are preferably crosslinked in the mixture. With a different specific energy input from a laser 2, only the adjacent myoblasts on the outer side of the ring are crosslinked in this preferred embodiment (Fig. 13 and 14)

In a further preferred embodiment it is provided that the non-crosslinked cells inside the vascular structure are removed by means of a laser.

Bioinks consisting of a particularly homogeneous cell type are also preferably crosslinked in order to print larger homogeneous tissue areas, for example.

In a preferred embodiment, the cells to be networked are included in a network that is being formed.

In a further embodiment, the cells themselves are networked with one another or with a network. To do this, they have a crosslinking molecule that is anchored in the cell membrane.

Due to the comparatively fast printing process, it is surprisingly possible to transfer the printed tissue relatively promptly to an incubator in which further maturation is triggered by environmental factors (eg chemical, molecular-biological, or flow stress).

Other crosslinkable inks are preferably printed at specific positions that carry support functions for the tissue, carry factors that are important for the physiology of the cell, or carry growth factors, markers, signaling molecules, receptors or other binding sites (key-lock principle).

Similar to the inkjet printer for photorealistic art prints, the printer head preferably has a larger number of nozzles that are adapted to the respective rheology of the ink. The printer also preferably has one or more sources of omnidirectional or directional electromagnetic waves, which initiate the crosslinking reactions.

In a preferred embodiment, a printer head for photorealistic art prints is used, which has been additionally modified with one or more sources for electromagnetic waves.

Patent claims

1. **3D printing** method for the production of tissues and organs having vascular structures by means of

(a) a droplet printer for photorealistic high-resolution prints and

(b) a device for applying electromagnetic waves,

the method including the steps of:

- providing at least one bio-ink and one capillary ink with cells and cross-linking molecules in the droplet printer;
- placing at least one drop of the bio-ink and the capillary ink on one reaction level, wherein the capillary ink comprises a special bio-ink capable of crosslinking only selectively to a limited extent in edge areas of the at least one drop or in areas adjacent to the at least one drop of the at least one bio-ink;
- bringing the electromagnetic waves into contact with the crosslinking molecules in the at least one drop at the reaction level; and
- activation of the cross-linking molecules by means of non-directional or directed movements of the electromagnetic waves in the at least one drop, whereby cross-linked structures are formed and vascular structures are thus obtained.

2. The method according to claim 1,

characterized in that

the at least one drop has a drop size between 1 fl (femtoliter) and 1 µl (microliter).

3. The method according to claim 1 or 2,

characterized in that

the capillary ink only crosslinks or forms a layer in an edge area of the at least one drop and that non-crosslinked or unbound components are eliminated.

4. The method according to claim 3,

characterized in that

crosslinking or layer-forming reactions in the edge area are caused by components in the capillary ink and the at least one bio-ink, or by the influence of the electromagnetic waves.

5. The method according to claim 3 or 4,

characterized in that

reactions in the edge area are caused by:

- thiol-ene reactions;
- cycloadditions;
- nucleophilic ring openings;
- self-assembly of molecules and particles;
- selective factors on the cells of the capillary ink and/or the at least one bio-ink; or
- other reactions with a similar effect.

6. The method according to any one of claims 1 to 5,

characterized in that

the at least one bio-ink includes molecules that support the physiology of the printed tissue or organ.

7. The method according to any one of claims 1 to 6,

characterized in that

the at least one bio-ink contains growth factors, transcription factors, signaling molecules, marker molecules and/or target molecules for later key-lock reactions.

8. The method according to any one of claims 1 to 7,

characterized in that

culture medium or blood flows through the printed vascular structures during printing.

9. The method according to any one of claims 1 to 8,

characterized in that

the reaction level is positioned in a bioreactor, which is connected to a blood circuit.

10. The method according to any one of claims 1 to 9,

characterized in that

the device for applying the electromagnetic waves is a UV lamp, a diode, a screen or a laser beam device.

11. Print pattern for 3D printing of tissues and organs having vascular structures,

characterized in that

the following properties are present:

- tissue plane composed of different types of inks, characterized in that some of the inks have undergone cross-linking or polymerisation,using a 3D printing method according to any one of claims 1 to 10, only in an edge area of at least one drop of one type of ink, in proximity to drops of other types of inks;

- tissue plane made up of various, ordered cell types, which are traversed by incised vessels (veins, arteries and capillaries) that are hollow on the inside;
- subdivision of larger tissues or organs into individual modules and simplification of a printed image for an economical printing process, wherein the printed image is obtainable from the 3D printing method according to any one of claims 1 to 10;
- printing of the individual modules independently of one another on the same or on different printers and subsequent gluing of the individual modules to a desired structure of a tissue or an organ, where each printer includes a droplet printer used in the 3D printing method according to any one of claims 1 to 10;
- the individual modules with at least one ascending and one descending branch for medium supply and discharge as well as fan-shaped capillaries in between, which form a microcirculation, which forms a smallest vessel unit;
- combination of micro circuits to form main vessels for a medium inlet and outlet when building larger structures from the individual modules;
- stabilization of the printed image by means of a biodegradable or resorbable printing foil, which delimits both printing sides in a Z-direction and has secondary and main connections for the main vessels.

12. Printing table of a droplet printer used in a 3D printing method according to any one of claims 1 to 10, the printing table for 3D printing of tissues and organs having vascular structures,

characterized in **that**

it is made up of the following components and has the following properties:

- a plane movable in the Z-direction of a medium-filled pressure space and having supply connections through which a printed image is supplied;
- a printer table base with adjustable medium inlet and outlet as well as connecting pins and magnetic attachment for the printing table;
- a changeable printing table with printing plate and printing film;
- pressure plate with medium inlet and outlet as well as tissue type-specific secondary connections;
- a pressure foil with secondary connections of the pressure plate for separating the tissue from the pressure plate;
- the printing film is preferably made of biodegradable or resorbable polymer;
- easy to put on and take off the printing table by means of a magnetic connection with a printer table foot of the printing table;
- connection pin of the printer table base as a connecting element between an outer area and the medium-filled pressure space (through the printing table);
- a pressure plate with a receiving opening for the connection pin of the printer table base;
- a replaceable silicone mat between the printer table foot and the printing table to separate and seal a printing space;
- simple connection and sealing of a medium supply via plug-in connection of the connection pin;
- an external device for maturing the printed image in an incubator, also consisting of a medium tray and an identical printer table base with a plug-in connection to accommodate the printing table with the pressure plate or, alternatively, only the pressure plate to supply the printed image;

- blood or another liquid adaptable to flow through the medium inlet and outlet instead of the medium-filled pressure space.

13. Printer head supply unit of a droplet printer used in a 3D printing method according to any one of claims 1 to 10, the printer head supply unit for 3D printing of tissues and organs having vascular structures, **characterized in that**

it is made up of the following components:

- a cell concentrator, in which cultured cells from a cell tank are concentrated to a specific cell concentration;
- an ink concentrate tank;
- a mixing unit with a cell counter, in which a required number of cells per volume and a required ink concentration are mixed according to specifications of a printing algorithm and forwarded to a printer head of the printer head supply unit, wherein the printing algorithm is provided by the 3D printing method according to any one of claims 1 to 10;
- the number of printer head supply units depends on and varies with the number of inks required;
- a printhead power unit of the printer head supply unit is controlled by the printing algorithm to mix a required ink composition and has a number of sensors that provide the printing algorithm with information about properties of inks currently being mixed, to which the printing algorithm reacts, wherein the inks are provided by the 3D printing method according to any one of claims 1 to 10.

14. Tissue incubator for maturing a printed tissue,

characterized in that

it has the following components:

- medium tank with openings for hoses for inflow and outflow of a medium to a printer table base of a printing table of a droplet printer used in a 3D printing method according to any one of claims 1 to 10 and a permanently installed base, on which the printer table base dimensioned and shaped identical to the droplet printer is exchangeably attached and on whose spigot the printing table together with a printing plate of the printing table, a printing foil of the printing table and the printed tissue, is placed by means of magnetic force (without silicone membrane).

15. Specific algorithm for calculating a printed image and organizing a 3D printing method according to any one of claims 1 to 10, the specific algorithm

characterized in that

- calculation of the printed image is carried out in relation to a type of cell used (type of ink), as well as in relation to a distance and size of capillaries, and arterial and venous structures, and that on one hand an optimal capillary supply and on the other hand anatomical conditions of respective tissue structure are simplified in a common, modular and economically structured printed image, as well as
- that the specific algorithm breaks down larger target structures into smaller individual modules that are printable independently of each other, and that
- the specific algorithm provides file information for 3D printing of a printing plate and a printing foil and makes the file information exportable from a computer program for used in another 3D printer and that
- the specific algorithm for organizing the 3D printing method according to any one of claims 1 to 10 receives and processes the following information:
    - information about anatomical and physiological data as well as dimensions of tissues and organs to be printed,

- information from a printer head supply unit according to claim 13 about filling levels of tanks, cell concentration in a reactor and in a cell concentrator and at its outlet, mixed volumes in a mixer, as well as information about processes in a printer head of the printer head supply unit,

- information about a position of a printing table according to claim 12, a medium fill level in a print room, a medium volume flow per unit of time in inlets and outlets in a printer table area, about a direction of the medium volume flow,
  - information about current dimensions of the printed image,
  - information about current performance of a printer table drive, as well as that the specific algorithm receives information about an activity of a source of electromagnetic waves;
- and that the specific algorithm sends the following signals to appropriate printer areas:
  - signals to individual components of the printer head supply unit to mix required ink components;
  - signals to pumps, which are used for a specific volume transport of inks in the mixer and in the printer head;
  - signals for organizing inflow and outflow of a medium on a way from a printer table foot of a printing table of a droplet printer used in a 3D printing method according to any one of claims 1 to 10 to vessels of the tissues and for organizing a flushing process in the capillaries;
  - signals for a required movement of the printing table;
- signals to the printer head for selective control of printer nozzles and for moving a printer head carriage;
- signals to the source of the electromagnetic waves;
- signals for optional pressure pauses during capillary rinsing.