CLAIMS

What it claimed is:

1. A process of preparing cell-cultured meat using a microfluidic 3D printing technology-based cell-cultured meat production device, wherein the process comprises:

Step 1: Preparing the biological ink required for microfluidic 3D printing for later use;

Step 2: Importing the printing instruction file into the printing device, loading the biological ink prepared in Step 1 into the sample injection system (4); connecting the sample injection system (4) to the inlet of the printing nozzle (1), which is the channel inlet of the microfluidic chip, and squeezing the biological ink through the pipe (43) into the printing nozzle (1), i.e., the microfluidic chip; after fibers are generated at the outlet of the microfluidic chip, selecting the instruction file to be printed and starting the entire device; the fibers generated at the outlet of the printing nozzle (1) are deposited on the loading platform (3) driven by the optical axes of the printing moving system (2) and are stacked and shaped according to the printing instruction path;

Step 3: Disassembling the loading platform (3) and performing crosslinking and curing treatment on the three-dimensional tissue obtained from the printing in Step 2, and then transferring it to the corresponding culture medium for proliferation and differentiation culture;

Step 4: Harvesting the matured three-dimensional tissue cultured in Step 3, washing to remove the culture medium, and performing food processing to obtain the cell-cultured meat;

The biological ink described in Step 1 is a hydrogel solution containing seed cells, or a combination of a non-adhesive cell material and the hydrogel solution containing seed cells; the non-adhesive cell material is able to be directly mixed with the hydrogel solution containing seed cells or can encapsulate the hydrogel solution containing seed cells; the hydrogel solution containing seed cells contains 30%-70% by volume of biological material and 0.01%-1% crosslinking agent, and balance is a basal medium containing a calcium salt and of $5\times10^{6\text{-}5}\times10^{8}$/mL of seed cells;

The device comprises a printing nozzle (1), a printing moving system (2), a loading platform (3), a sample injection system (4), and a base (7); the printing moving system (2) is placed on the base (7) and consists of multiple movable optical axes, with the printing nozzle (1) fixed on one of the movable optical axes and the loading platform (3) connected to another movable optical axis; the sample injection system (4) is connected to the printing nozzle (1), and the printing nozzle (1) is a microfluidic chip capable of manipulating, processing, and controlling trace liquids or samples in its channel.

2. The process according to claim 1, wherein the printing moving system (2) comprises an x-axis movable optical axis (21), a z-axis movable optical axis (22), and a y-axis movable optical axis (23); the z-axis movable optical axis (22) and the y-axis movable optical axis (23) are fixed on the base (7), the x-axis movable optical axis (21) is connected to the z-axis movable optical axis (22), the printing nozzle (1) is fixed on the x-axis movable optical axis (21), and the loading platform (3) is connected to the y-axis movable optical axis (23).

3. The process according to claim 2, wherein the printing moving system (2) consists of moving axes capable of driving the printing nozzle (1) to move in two directions on the x-axis movable optical axis (21) and the z-axis movable optical axis (22), and capable of driving the loading platform (3) to move on the y-axis movable optical axis (23), and a movable coordinate system configured for the printing moving system can be any one of a Cartesian coordinate system, a triangular coordinate system, a polar coordinate system, or a planar joint coordinate system.

4. The process according to claim 2, wherein the loading platform (3) has a detachable structure, and is assembled into the printing moving system (2) and is connected to the y-axis movable optical axis (23) for assembly line printing; and the loading platform (3) is made of copper, aluminum, iron, steel, alloy, glass, ceramic, or carbon fiber plates.

5. The process according to claim 1, wherein the sample injection system (4) comprise a sample loader (41), a sample injection pump (42) and a pipe (43), the sample injection pump (42) is placed on a horizontal table top, the sample loader (41) is fixed on the sample injection pump (42), one end of the pipe (43) is connected to an outlet of the sample loader (41), the other end of the pipe is connected to a sample inlet of the printing nozzle (1), and a feeding manner of the sample injection system (4) comprises piston-type extrusion, pneumatic extrusion or screw-type extrusion.

6. The process according to claim 1, wherein a printing control display system (5) and a data transmission system (6) are embedded and installed in the base (7); and the data transmission system (6) is connected to the printing control display system (5) wirelessly or through a data cable, and the printing control display system (5) is connected to the printing moving system (2) wirelessly or through a data cable.

7. The process according to claim 6, wherein the printing control display system (5) is mainly configured to control printing leveling, select a printing program, issue a printing instruction and perform a position adjustment of the printing moving system (2); the data transmission system (6) is configured to transmit a printing instruction file into a microfluidic 3D printing device; and a data transmission form of the data transmission system (6) comprises USB transmission, memory card transmission or computer transmission.

8. The process according to claim 6, wherein after the printing control display system (5) and the data transmission system (6) are embedded in the base (7), they are powered together with the base (7).

9. The process according to claim 1, wherein the microfluidic chip on the printing moving system (2) can be flexibly replaced to perform integrated printing according to different production demands.

10. The process according to claim 9, wherein one or more microfluidic chips can be integrated on the printing moving system (2) as the printing nozzles (1), and when a plurality of the microfluidic chips are integrated on the

printing moving system, microfluidic chips with a same channel structure or different channel structures can be used.

11. The process according to claim 1, wherein the microfluidic chip can be integrated on the printing moving system (2) by means of clamping, snap-fitting, plugging, magnetic attraction, tenoning, riveting, threaded connection, or bayonet connection.

12. The process according to claim 1, wherein materials for making the microfluidic chip comprise one or more of crystalline silicon, polydimethylsiloxane, quartz, polyphthalamide, polymethyl methacrylate, polycarbonate, polystyrene, epoxy resin, acrylic acid, rubber, and fluoroplastic; and a method for making the microfluidic chip comprises glass capillary assembling, machining, etching, or molding.

13. The process according to claim 1, wherein the microfluidic chip can be of a single-channel type, a coaxial nested type, or a multi-channel parallel type.

14. The process according to claim 1, wherein the microfluidic chip can, based on microfluidic chips with different structures, generate solid, "shell-core", hollow, multi-component, spiral, and string-bead fibers for microfluidic 3D printing.

15. The process according to claim 1, wherein the non-adhesive cell material in the biological ink is any one or more of sodium alginate, chitosan, pectin, carrageenan, and gellan gum; and a concentration of the non-adhesive cell material solution is 10-50 mg/mL.

16. The process according to claim 1, wherein the seed cells in the biological ink are derived from any one or more of a pig, cattle, sheep, a chicken, a duck, a rabbit, a fish, a shrimp, and a crab; and the seed cells are one or more of muscle stem cells, myoblasts, myosatellite cells, muscle precursor cells, bone marrow-derived mesenchymal stem cells, adipose-derived mesenchymal stem cells, induced pluripotent stem cells, cardiomyocytes, adipose stem cells, adipose precursor cells, bone marrow-derived adipose cells, fibroblasts, smooth muscle cells, vascular endothelial cells, epithelial cells, neural stem cells, glial cells, osteoblasts, chondrocytes, liver stem cells, hematopoietic stem cells, stromal cells, embryonic stem cells, or bone marrow stem cells.

17. The process according to claim 1, wherein the biological material is one or more of collagen, recombinant collagen, gelatin, matrigel, hyaluronic acid, silk protein, elastin, spider silk protein, fibrin, fibrinogen, silk fibroin, laminin, fibronectin, integrin, cadherin, nestin, decellularized extracellular matrix, chondroitin sulfate, heparin, keratan sulfate, dermatan sulfate, heparan

sulfate, keratin, keratin sulfate, cellulose, polymerase, carboxymethyl cellulose, polylactic acid, polyvinyl alcohol, lecithin, nanocellulose, soy protein, pea protein, gluten protein, rice protein,

peanut protein, yeast protein, fungal protein, wheat protein, potato protein, corn protein, chickpea protein, mung bean protein, seaweed protein, almond protein, or quinoa protein.

18. The process according to claim 1, wherein the basal medium used in the biological ink is one or more of F-10, DMEM, MEM, F-12, DMEM/F-12, DMEM/F-12 GlutamMAX^{TM}, F-12K, RPMI 1640, IMDM, L-15, 199, MCDB 131, LHC, or McCoy's 5A.

19. The process according to claim 1, wherein the crosslinking agent used in the biological ink comprises any one or more of NaOH, KOH, $NaHCO_3$, HEPES balanced salt solution, EBSS balanced salt solution, HBSS balanced salt solution, PBS, and DPBS, and transglutaminase, tyrosinase, laccase, lysyl oxidase, polyphenol oxidase, catalase, thrombin or genipin.

20. The process according to claim 1, wherein the crosslinking and curing treatment in the step (3) preferably comprises one or more of temperature-induced crosslinking, electrostatic interaction crosslinking, ion crosslinking, and enzyme crosslinking.

21. The process according to claim 1, wherein in the step (3), the proliferation culture medium comprises the basal medium with a volume ratio of 79-89%, 10-20% of fetal bovine serum, and 1% penicillin-streptomycin, and 1-10 ng/mL alkalic fibroblast growth factor is added into the solution; and the differentiation culture medium comprises the basal medium with a volume ratio of 94-97%, 2-5% horse serum and 1% penicillin-streptomycin.

22. The process according to claim 15, wherein the food processing in the step (4) comprises preprocessing and cooking, and the preprocessing comprises one or more of cleaning, seasoning, color enhancement, modeling, sensory quality modification, and the like, and the cooking comprises frying, deep frying, boiling, steaming, baking, and the like.