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3D bioprinting of hybrid cultured meat from rabbit cells and sunflower protein

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Abstract:
A quarter of the world's population has no access to safe foods of high quality due to the inability of traditional agriculture to meet the growing needs. Therefore, cultivated meat produced from a large mass of animal cells in a laboratory is becoming a promising alternative to animal products. In this study, we aimed to develop a technology for obtaining a hybrid cultured meat product from rabbit cells, sodium alginate, and sunflower protein, as well as to analyze its morphological and functional characteristics.

We used rabbit stem cells isolated from the greater omentum and exposed to lipogenic and myogenic differentiation, as well as rabbit skin fibroblasts. The cells were placed in a hydrogel of sodium alginate and sunflower protein and cultured for 72 h to biofabricate tissue constructs by using 3D bioprinting. Confocal and transmission electron microscopy was applied to analyze the morphological and functional characteristics of the cells in the constructs.

Using 3D bioprinting, we obtained tissue constructs of 30×40×3 mm from rabbit cells, sodium alginate, and sunflower protein. According to confocal microscopy, the cells in the tissue constructs remained viable for at least 72 h. Transmission electron microscopy showed that the cells formed tight junctions and were metabolically active for at least 72 h, with fibroblasts secreting procollagen and lipoblasts secreting lipid droplets.

The resulting cellular meat was obtained from a combination of fibroblasts, lipocytes, and myogenic cells, as well as two ink components. The cellular meat product was safe and ready for consumption.

Keywords: Cultured meat, tissue construct, 3D bioprinting, stem cells, rabbit, vegetable protein

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INTRODUCTION

Over the last 200 years, the world's population has increased eightfold to reach about 8 billion people in 2022. By the end of the 21st century, this figure may grow to 10 billion [1].

According to the UN, about 690 million people worldwide (8.9% of the world population) currently suffer from hunger, and about 2 billion people do not have access to safe and good-quality food [2].

Meat is an essential part of a balanced human diet. Due to its properties, meat has played a decisive role in human evolution and contributed to the development of many economic sectors [3]. Today's animal husbandry, for example, boasts 1.4 billion cattle, 1.1 billion sheep, and 18.6 billion chickens. Farm animals are dominant among all terrestrial vertebrates in terms of their numbers and biomass [4]. However, instead of eliminating hunger, their increasing production leads to a number of global problems. These include higher greenhouse gas emissions, unsustainable use of land and water, antibiotic resistance, and a negative impact on biodiversity [5]. Moreover, animal husbandry is also associated with

some ethical problems, such as inhumane euthanasia, mistreatment of animals, and hard, low-paid manual labor under unsatisfactory conditions [6]. These problems contradict modern principles of sustainable development [7].

In response to these global challenges, food scientists are developing technologies to produce alternative food products that can replace animal protein. They include products based on textured plant protein. Originating in the mid-20th century, they have now taken a significant share of the world market [8]. There are also techniques for producing alternative protein from microalgae [9] or insects, which are part of traditional cuisine in some parts of the world [10]. A relatively new idea is to obtain protein from bacteria, mold, and yeast-like fungi, including fermentation of waste from plant and animal products [11].

However, according to surveys and sensory evaluations, alternative protein products are not yet seen as a complete meat substitute. Despite many years of marketing, their consumption is still insignificant compared to the increasing consumption of meat [12–14].

Cultured meat is a technology that could potentially overcome this misbalance. Put forward by different scientists at different times, this technology was systematically developed by Mark Post, who also proposed its practical implementation [15, 16]. The idea is based on modern achievements in stem cell technology and tissue engineering. In particular, it involves growing edible tissues from donor animal cells under laboratory conditions. Within ten years of the first tasting, 55 companies in 19 countries started to produce cultured meat [17]. Scientists working in cell technologies aim to optimize standard laboratory procedures for the industrial production of cell mass. They replace fetal bovine serum in cell culture media with synthetic or plant analogues and develop methods for giving the cell mass a dense texture that is sensorily similar to that of traditional meat [13, 14, 16, 17].

Since it is technologically difficult to create a dense texture, most cultured meat produced today is minced [13, 16]. Despite its wide use in the food industry, minced meat is mainly used in various convenience foods, which also contain large amounts of fat, salt, and sugar. Such components and flavors are added to improve the taste of cultured meat, which is still inferior to that of traditional meat. This goes against the principles of healthy eating. Therefore, the nutritional profile of cultured meat products needs to be optimized, for example, by using plant protein.

Protein from plant processing waste (e.g., legumes or sunflower) is a cheap source of essential nutrients [18–20]. Since high cost is one of the main obstacles for a widespread use of cultured meat [21, 22], using plant protein can significantly reduce the cost of hybrid meat products.

The 3D layer-by-layer printing technology is increasingly used in various fields. It can create complex three-dimensional structures with high accuracy and reproducibility based on a digital template. This is one of the leading methods in tissue engineering, where it is known as 3D bioprinting [23]. It can be used to create three-dimensional cellular and tissue constructs and simple organs [24]. The use of 3D printing in the food industry is referred to as foodprinting [25]. Potentially, a combination of foodprinting and 3D bioprinting can create meat products with a texture as close as possible to that of meat. This can reduce sensory differences between cultured minced meat from cellular mass, plant protein, and traditional meat [26]. 3D printing also allows for customizing the shape of the final product and varying the ratio of cellular and plant protein mass, which opens up opportunities for a personalized approach [27]. Cellular meat is produced from cultures of multipotent mesenchymal stem cells (MMSCs) and fibroblasts. They are usually obtained from a donor animal by means of a lifetime tissue biopsy. MMSCs are differentiated into myoblasts and adipocytes, followed by cell culturing until the required mass is obtained [28].

In this study, we aimed to develop a technology for producing a hybrid cultured cell product from rabbit cells, sodium alginate, and sunflower protein, as well as to conduct its morphological and functional analysis. To achieve this aim, we set a number of objectives (Fig. 1), namely to: 1) select a donor animal; 2) isolate stable cultures of MMSCs and fibroblasts from the donor, and conduct the myogenic and adipogenic differentiation of MMSCs; 3) manufacture a hydrogel based on sodium alginate and sunflower protein; 4) biofabricate a three-dimensional construct using 3D bioprinting with bioink made from the hydrogel and cells; 5) cultivate the resulting construct in a culture medium for 72 h; and 6) conduct a morphological and functional analysis of the cells in the printed construct using confocal laser scanning microscopy and transmission electron microscopy.

STUDY OBJECTS AND METHODS

Obtaining tissue from a donor animal. An adult male rabbit *Oryctolagus cuniculus domesticus* of the Soviet Chinchilla breed was used as a donor animal. The animal was kept in a vivarium in accordance with the internal regulations of the local ethical committee of Don State Technical University and the laws of the Russian Federation. To isolate multipotent mesenchymal stem cells (MMSCs), 2-g fragments of adipose tissue were obtained from the greater omentum by laparoscopic biopsy. Fibroblasts were isolated from the abdominal skin biopsy samples.

Isolating and cultivating MMSCs. MMSCs were isolated from a fragment of adipose tissue of the greater omentum under sterile conditions in a biological safety box. For this, we followed the protocol proposed by Bunnel *et al.* [29]. First, we incubated the biopsy samples in a 0.2% solution of collagenase from crab hepatopancreas (Biolot, Russia) in Dulbecco's phosphate-buffered saline (DPBS) (Biolot, Russia) for 60 min at 37°C and constant shaking. Then, we filtered the resulting suspension through a cell sieve and allowed the cells to sediment by centrifugation.

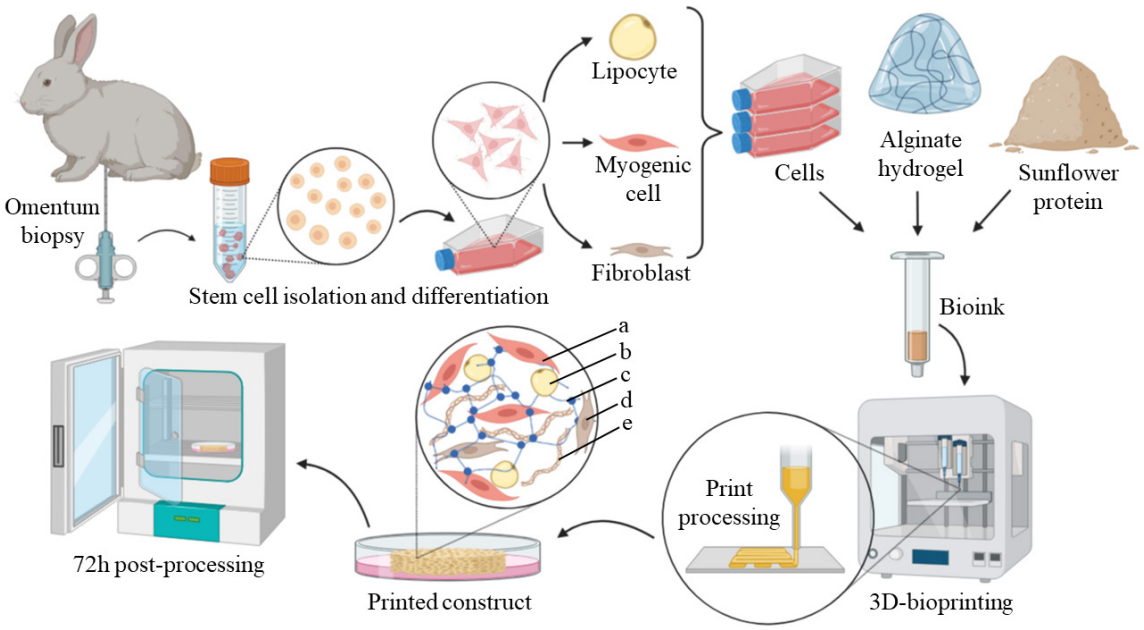


Figure 1 The main stages of production of a hybrid cultured meat product (a – myogenic cell, b – lipocyte, c – Ca^{2+} ionic crosslinked alginate, d – fibroblast, and e – sunflower protein)

Then, we placed the resulting cell mass into culture flasks with the DMEM/F12 medium (Biolot, Russia) and added 10% fetal bovine serum (Biolot, Russia) and 1 ng/mL recombinant basic human fibroblast growth factor (FGF2, Sigma-Aldrich, USA). After 48 h, the medium was changed to remove non-adherent cells. The resulting cell culture was maintained for five passages. Antibiotics and antimycotics were not used during cell cultivation. A 0.25% trypsin-Versene (1:1) solution (Biolot, Russia) was used for cell disaggregation during the passages.

Differentiating MMSCs. To induce adipogenic differentiation, we used a DMEM medium with a high glucose content and stable L-glutamine (Biolot, Russia), 10% fetal bovine serum (Biolot, Russia), 0.1 µmol dexamethasone (D4902 Sigma-Aldrich, USA), 0.45 µmol isobutylmethylxanthine (I5879 Sigma-Aldrich, USA), 170 nmol insulin (I5500 Sigma-Aldrich, USA), and 0.2 µmol indomethacin (I7378 Sigma-Aldrich, USA) [30]. On the 10th day of cultivation, the medium was replaced with a complete DMEM medium for further use. The MMSC culture cultivated in a complete DMEM medium without any inducers was used as a differentiation control.

Lipid droplets detected in the cell cytoplasm during microscopy were taken as a specific differentiation marker [30].

To induce myogenic differentiation, we used a DMEM medium with a high glucose content and stable L-glutamine (Biolot, Russia), 10% fetal bovine serum (Biolot LLC, Russia), 10 mM 5-azacytidine (Sisco Research Laboratories, India), and 50 µmol hydrocortisone (H6909 Sigma-Aldrich, USA) [31]. On the 15th day of cultivation, the medium was replaced with a complete DMEM medium for further use. The MMSC culture cultivated in a complete DMEM medium without any inducers was used as a differentiation control.

Cell fusion with the formation of multinucleated myotubes detected by microscopy was taken as a specific differentiation marker [32].

Isolating fibroblasts. Fibroblasts were isolated from the skin biopsy samples under sterile conditions in a biosafety box according to the protocol proposed by Abade dos Santos [33]. To prevent microbial contamination, the tissue fragments were washed five times in a DMEM medium with a solution of antibiotics and antimycotics (200 units/mL penicillin, 200 µg/mL streptomycin, 0.5 µg/mL amphotericin B, and 50 µg/mL gentamicin) with vigorous shaking. The medium was changed every 5 min.

The tissue fragments were sterilely cut with scissors, placed in a trypsin solution preheated to 37°C for dissociation, and incubated at 37°C for 10 min. After that, the solution was centrifuged at 150 g for 10 min. The sedimented cells were resuspended in a DMEM medium with 10% fetal bovine serum and a solution of antibiotics and antimycotics (100 units/mL penicillin, 100 µg/mL streptomycin, 0.5 µg/mL amphotericin B (Gibco, USA), and 25 µg/mL gentamicin (Biolot, Russia)). Then, the suspension was transferred into culture flasks and incubated at 37°C with 5% CO_2 for 4 h, after which the flasks were washed for a new portion of the medium to be added. After 2 h, washing was repeated and the medium was changed. Subcultivation was carried out when the density of the cell layer reached 90%, with no antibiotics or antimycotics used in the further passages.

Sunflower protein. Dry sunflower protein concentrate with a protein content of 840 g/kg (Kjeldahl method) was provided by MEZ YUG RUSI, Russia.

Bioink preparation. A mixture of sodium alginate and sunflower protein concentrate was used to prepare the bioink. The components were sterilized under ultraviolet light in a biosafety box for 30 min. Then, the components were dissolved in a Dulbecco's phosphate-buffered saline (DPBS) solution without calcium and magnesium (Gibco, China). First, 30 mg/mL of sodium alginate (Sigma-Aldrich, Germany) was dissolved in the DPBS solution on a rotary shaker at room temperature for 1 h. Then, sunflower protein concentrate was added to the solution at a concentrate:alginate ratio of 3:1 dry weight basis. The mixture was homogenized and kept for 2 h at room temperature. Then, a 5-mL syringe was filled with 3 mL of hydrogel and centrifuged for 3 min at 3000 rpm to remove air bubbles.

Immediately before printing, fibroblasts, lipocytes, and myosatellite cells (1:1:1) were pelleted by centrifugation and resuspended in the hydrogel (2×10^6 cells/g) by mixing between two syringes connected by an adapter. Finally, the hydrogel with cells was transferred into the injector, which was placed in the bioprinter head for printing to begin.

3D bioprinting and bioprinter characteristics. For 3D bioprinting, we developed a hydraulically driven print head in the form of a peristaltic pump and a stepper motor. It was installed on an Anet A8 FDM printer. Such a drive allowed us to keep the extrusion speed and the speed of the printer actuators in accordance to each other, as well as to implement a bioink retract procedure at the end of each layer to improve the print quality. Slic3r in the Repitier Host 3D printer control software package (Hot-world GmbH and Co, Germany) was used as a slicer with the following parameters: object's dimensions – 30×40×3 mm, layer height – 0.2 mm, number of perimeters – 0, number of solid layers at the bottom – 0, number of solid layers at the top – 0, filling – 8%, filling type – rectilinear, filling angle – 90°, filling printing speed – 10 mm/s, 25 G nozzle diameter – 0.2 mm, filament diameter – 1.2 mm, extruder feed multiplier – 1.3.

Printing was performed in laminar flow in a biosafety box, and all procedures were conducted at room temperature. Material strands were deposited in 60-mm Petri dishes using air as a plotting medium. After plotting, the scaffolds were transferred into 100 mg/mL of $CaCl_2$ solution and incubated for 10 min to crosslink the alginate component. After crosslinking, the printed construct was transferred to Petri dishes with a DMEM medium and placed in a CO_2 incubator at 37°C for 72 h.

Confocal laser scanning microscopy. Fragments of tissue constructs were incubated in a solution with Sytox Green Stain dye (ThermoFisher Scientific, USA, dilution 1:1000), washed in phosphate buffer, embedded in a medium that prevents signal fading (Abberior, Germany), covered with a cover glass, and viewed in an Abberior Facility Line inverted confocal laser scanning microscope (Abberior Instruments GmbH, Germany). To visualize a 3D object, it was scanned along the Z-axis with a step of 200 nm at a pixel size of 40 nm. The 3D model was constructed using the ImageJ program.

Transmission electron microscopy. Fragments of tissue constructs were fixed in 2.5% glutaraldehyde (Aurion, USA), washed in phosphate buffer, and additionally postfixed in a 1% OsO_4 solution in phosphate buffer for 1.5 h. Then, the cells were dehydrated in alcohols of increasing concentration and absolute ethanol, treated in three changes of propylene oxide, and embedded in epoxy resin based on Epon-812. Semi-thin and ultrathin sections were made using an EM UC 7 ultramicrotome (Leica, Germany) and an ultra 45° diamond knife (Diatome, Switzerland). Semi-thin sections were stained with methylene blue and viewed under a Leica 6000 light-optical microscope (Leica, Germany). Ultra-thin sections were contrasted with uranyl acetate and lead citrate and viewed in a Jem-1011 electron microscope (Jeol, Japan) with an accelerating voltage of 80 kV.

RESULTS AND DISCUSSION

We chose the rabbit as a cell donor because this animal is commonly used in both meat farming and scientific research. Biopsy allowed us to save the life of the donor animal, which is one of the most important principles in the concept of cultured meat. However, we should also consider restrictions that some religions impose on the consumption of certain parts of a living animal. In Islam or Judaism, for example, an animal has to be slaughtered in a ritual for the product to be kosher or halal [34, 35]. This is an important issue for a large number of potential consumers of cultured meat. Noteworthily, the use of a rabbit as a cell donor makes the resulting product non-kosher [36]. Yet, Judaism allows the consumption of cultured meat produced from the cells of kosher animals slaughtered in a ritual manner [35].

The protocols used for cell isolation and differentiation allowed us to obtain stable cell cultures (Fig. 2), which were subcultured during 25 passages for fibroblasts and lipocytes and 12 passages for myogenic cells.

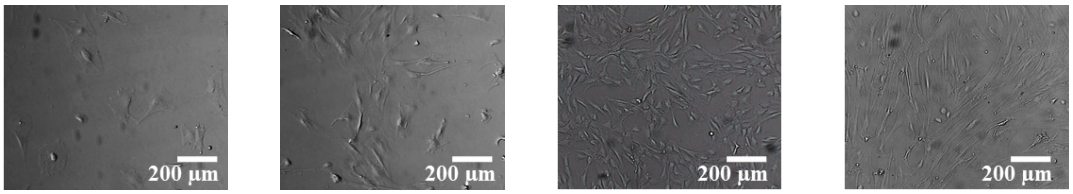


Figure 2 Cell cultures used in this study: (a) MMSCs; (b) fibroblasts; (c) lipocyte; and (d) myogenic cells

Sodium alginate is a standard component of bioink in 3D bioprinting. It is widely available and its hydrogels have acceptable characteristics for cell growth in 3D printed constructs. Due to its ability to cross-link in the presence of calcium ions, sodium alginate contributes to stable constructs [37, 38]. It is a registered food additive (E401) that can be used not only as a thickener and stabilizer, but also as a functional component in nutrition [39, 40].

Sunflower protein concentrate is a second component of bioink. It is non-toxic to cells, has acceptable printability (Fig. 3a), and is widely used in the food industry. In addition, due to its texture and density, plant protein can give the final product familiar sensory properties expected from meat [41].

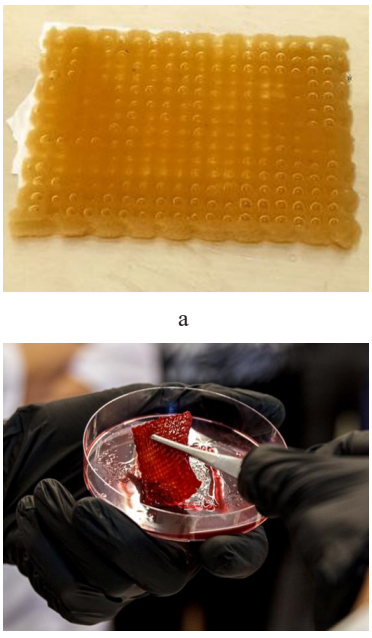
The color of a product is an important sensory parameter. Although it is not a reliable indicator of safety or quality, consumers often associate the meat's color with its healthiness, which affects their purchasing decision [42]. The technology we used in this study did not allow us to obtain a large amount of myoglobin, which is mainly responsible for the meat's color. Therefore, we used raw beet juice to give the final product a "meaty" color without affecting its structure and integrity. Particularly, the constructs were immersed in raw beet juice for 5 min after post-processing (Fig. 3b).

For sensory evaluation, the samples were fried on both sides in an open frying pan using refined and deodorized vegetable oil at 200 °C for 4 min, without adding spices or flavorings. During frying, the product's color changed from red to yellowish-brown as a result of the Maillard reaction. The panelists described the taste, aroma, and texture of the product as "similar to a meat product".

In semi-thin section preparations stained with methylene blue, fragments of the construct (after 72h of post-processing) consisted of spindle-shaped cells with elongated outgrowths, with moderately stained cytoplasm distributed around the nucleus (Fig. 4a). The cells were arranged in the form of rounded rosettes and clusters visibly contacting one another. The nuclei were oval and round, with finely dispersed chromatin, mostly large and small (Fig. 4b). Some fragments had outwardly empty lumens and others were filled with cytoplasmic outgrowths of the surrounding cells.

Thus, the cells of the constructs under study had the features of young fibroblasts and lipoblasts actively synthesizing proteins – cell growth and differentiation factors or intercellular components (collagen, elastin, glycosaminoglycans, proteoglycans, and multi-adhesive glycoproteins).

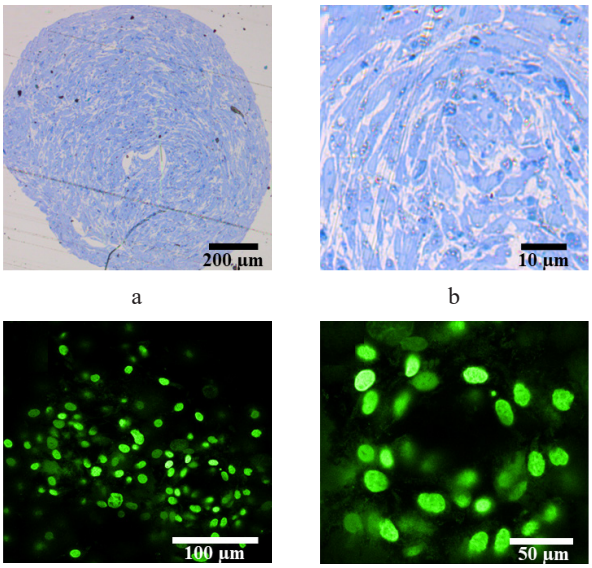
Immunofluorescence images of tissue construct fragments were obtained to determine if the cell nuclei were damaged. The samples prepared for immunofluorescence had nuclear rounded groupings resembling a spheroid. The nucleus of such cell groupings looked like a conglomerate of several dozens of cells circling as a crown around the center (Fig. 4c). The nuclei of such cells had an oval, slightly elongated shape with clear contours and uniform intranuclear distribution of nucleic acids (Fig. 4d).



a

b

Figure 3 The printed product: (a) immediately after printing; (b) after 72 h of post-processing (the product is colored with beetroot juice to look more familiar and is ready to cook)



a b c d

Figure 4 Light and confocal laser microscopy of the printed construct cells after 72 h of postprocessing: (a) transverse semi-thin section of the fragment stained with methylene blue (magnified 100×); (b) a conglomeration of cells inside the construct (magnified 400×); (c) cell nuclei inside the construct stained with Sytox Green (magnified 400×); and (d) enlarged section C, with a circular growth of cell nuclei of an oval regular shape with uniform non-condensed chromatin (magnified 600×)

According to the immunofluorescence and confocal microscopy, the cell nuclei were fragmentated and transformed, rather than damaged (Fig. 5).

The ultrastructural examination showed that the cells with ultrastructural features of fibroblasts and lipoblasts had spindle-shaped, elongated, and triangular bodies with branched thin outgrowths alongside, as well as small irregular cytoplasmic outgrowths. The cells were densely adjacent to each other, with fragments of direct contact between the membranes of the bodies and the outgrowths (Fig. 6).

Both fibroblasts and lipoblasts contained oval nuclei with finely dispersed chromatin. Almost every cell had one or two large nucleoli, which indicated fibroblast activity. In some cases, the cell nuclei had a curved shape due to invaginations and bulging of the karyolemma. The cytoplasm of fibroblasts was sufficiently saturated with organelles, indicating a high level of intracellular metabolism. Also visible were small oval and round mitochondria in large numbers, rough endoplasmic reticulum (RER), Golgi complexes, lysosomes, centrioles, and multi-vesicular bodies. The lipoblasts had multiple inclusions of round homogeneous osmiophilic lipid droplets of various diameters.

The developed network of the endoplasmic reticulum (rough or granular) and the Golgi complexes indicated an active synthesis of proteins, such as collagen. The endoplasmic reticulum occupied most of the cytoplasm. It looked like stacks of parallel-packed and twisted tubes, as well as rounded and elongated membrane cisterns with signs of expansion, which were dotted with ribosomes on the side facing the cytoplasm. The cytoplasm itself was saturated with individual ribosomes that were not attached to the membrane.

High magnifications also showed the activity of the Golgi complexes involved in the procollagen synthesis (Fig. 7a). Clearly distinguishable were both dictyosomes (transport vesicles) and spherical expansions limited by the membrane and having a smooth surface. Some expanded cisterns of the Golgi complex contained osmiophilic dense material, which might be procollagen molecules (tangled threads). Then the contents of the cylindrical vesicles condensed and turned into secretory granules and vacuoles. They contained the secreted product in concentrated form on the side facing the plasma membrane of the cell. The presence of a large number of Golgi complexes was also a sign of high secretory activity of the cells.

Specialized intercellular tight junctions detected in the cultured meat prototype were an important indicator of intercellular interaction (Fig. 7a, b). A tight junction formed between two dense osmiophilic areas where two cell membranes contacted with each other. At a certain plane of section, osmiophilic stripes were interrupted by a narrow, light intercellular gap that was parallel to them. Such contacts act as highly specialized selective barriers that provide direct contact between the proteins of two adjacent plasma membranes and allow groups of cells to function as structural units. In our prototype of cellular meat, tight junctions were detected between the cells of the same phenotype: fibroblast cells (fibroblast/fibroblast) and lipoblast cells (lipoblast/lipoblast) (Fig. 7a, b).

We managed to reduce the cost of our product by adding sunflower protein and sodium alginate to replace up to 60% of the cell mass, the most expensive component in this technology. At the time of the study, our product cost us $400/100 g. However, it cannot be considered the final cost since we only aimed to demonstrate a possibility of producing a cultured product from rabbit cells in laboratory conditions. According to our estimates, scaling this technology up by using bioreactors and serum-free media for cell cultivation will allow us to reach a cost of $60/kg for the final product at the current level of technological development.

Noteworthily, the technology of cultured meat is only beginning to enter the market, with only the USA and Singapore officially approving its sale [44]. However, since 2013, as much as $3.1 billion has been invested in the companies developing the cultured meat technology, their number reaching 174 in 2023 [45]. This shows high potential of this technology.

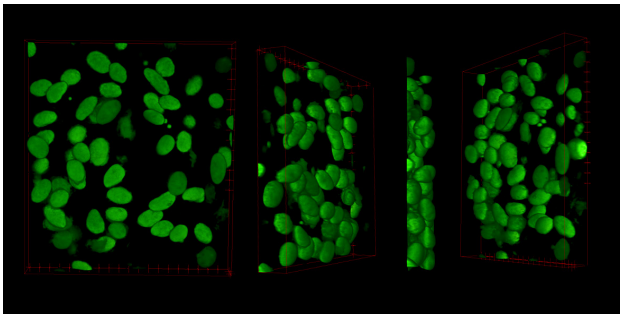


Figure 5 Confocal laser scanning microscopy of the construct's fragment stained with Sytox Green (3D reconstruction in ImageJ [43])

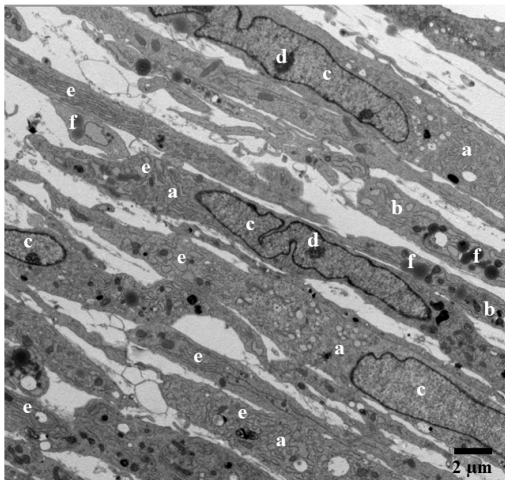
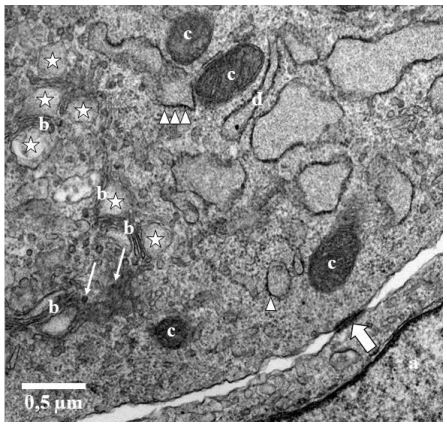
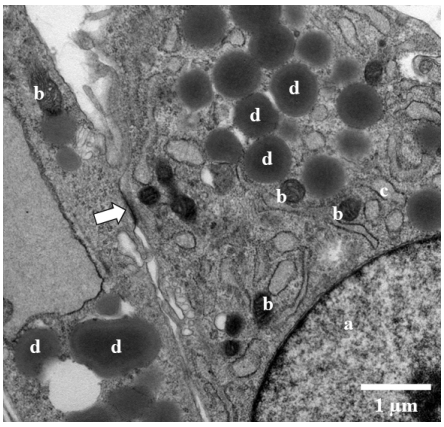


Figure 6 Transmission electron microscopy of the construct cells: a – fibroblast; b – lipoblast; c – nucleus; d – nucleolus; e – rough endoplasmic reticulum; and f – lipid droplets. Magnified 10 000×



Legends: a – nucleus; b – Golgi complex;
c – mitochondrion; d – rough endoplasmic reticulum.

a



Legends: a – nucleus; b – mitochondrion;
c – rough endoplasmic reticulum; d – lipid droplet.

b

Figure 7 Transmission electron microscopy showing cell proliferation within the printed construct during postprocessing:
(a) the ultrastructure of an active fibroblast region (magnified 80 000×). The Golgi complex is in the center of the micrograph, with spherical expansions (asterisks) in addition to the Golgi stacks. Some dilated Golgi cisternae contain osmiophilic dense material in the form of tangled filaments, which may be procollagen molecules. The content of the cylindrical vesicles condenses and is subsequently converted into secretory granules (thin arrows). Two fibroblasts are connected by a tight junction (thick arrow). Ribosomes (triangles) are attached to the rough endoplasmic reticulum.
(b) the ultrastructure of two lipoblasts connected by a tight junction (arrow) (magnified 40 000×).

We believe that 3D printing is hardly promising for commercial production due to its technical complexity. We see further development of the hybrid cultured meat technology in producing a cell mass cultivated on microcarriers in a bioreactor. Texturates of plant proteins, such as sunflower, can be used as microcarriers. This way we can obtain a product that resembles minced meat and, in the future, produce ready-to-eat products from it. However, 3D bioprinting can be used to produce cultured meat products for personalized nutrition.

CONCLUSION

In this study, we bioengineered a construct consisting of differentiated rabbit cells, alginate hydrogel, and sunflower protein by using 3D bioprinting. We showed a possibility of producing a cellular meat product from a selected combination of fibroblasts, lipocytes, and myogenic cells, as well as two ink components. According to confocal laser microscopy and electron microscopy, the cells in the construct remained viable, functionally active, and dividing for more than 72 h.

The product developed in our study is safe and ready to eat. We proved that cultured meat can be created from rabbit cells and biofabrication techniques can make the product's structure close to that of traditional meat.

Using components such as alginate and sunflower protein can reduce the cost of the final product and vary its nutritional properties and sensory qualities.

CONTRIBUTION

S.N. Golovin developed the study concept and design and was involved in the cell isolation and cultivation, bioink preparation, 3D bioprinting, and writing the manuscript (drafting, reviewing, and editing). E.Yu. Kirichenko managed the project, developed the study concept and design, prepared the samples for confocal laser scanning microscopy, imaging, and transmission electron microscopy, as well as contributed to writing the manuscript (drafting, reviewing, and editing). M.M. Khanukaev assembled the 3D bioprinter and was involved in 3D bioprinting and writing the manuscript (drafting, reviewing, and editing). A.K. Logvinov prepared the samples for transmission electron microscopy and contributed to writing the manuscript (drafting, reviewing, and editing).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this publication.

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