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This paper presents the method of magneto-acoustic biofabrication of a bioengineering construct from tissue spheroids. It allows creating tissue constructs of tubular shapes. Collected together in a magneto-acoustic trap, tissue spheroids contact with each other and thus fuse to form a 3D tissue construct. The magnetic system was made of two oppositely oriented permanent magnets with an empty space between them. A cylindrical ultrasonic transducer was placed into this space, and a container with tissue spheroids was placed inside the piezoceramic cylinder. The construct was formed in the region, where magnetophoretic forces in the vertical direction compensated gravity, and due to the magnetic gradient in the horizontal plane, the tissue spheroids moved towards each other, levitating above the bottom of the container. The piezoelectric transducer created standing cylindrical ultrasonic waves. The acoustic radiation force acted from the antinode to the node, forming a ring of tissue spheroids. Holding them in this trap for 18–20 hours led to the merging of spheroids into a solid ring-shaped living construct. Changes of the wave frequency and amplitude made it possible to manipulate the size and width of the resulting tissue ring.

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1. INTRODUCTION

Currently, there is a rapid development of tissue engineering and its course - biofabrication. This direction is aimed at growing artificial tissues from living cells or tissue spheroids (cellular agglomerates). One of the last biofabrication goals is to create three-dimensional tissue constructs that would have the same functions as human tissues and organs. The existing approaches are not entirely successful, as they often allow creating only a two-dimensional structure. On the one hand, solving this problem in 3D would allow us to test drugs and medical devices more efficiently, because flat and bulk tissue structures behave differently even consisting of the same cell type. On the other hand, this would let us create organ constructs that perform the functions of a real human organ. Since there is a strong lack of donor organs for transplantation, the biofabrication of an organ construct would be a solution to a global clinical problem. Recently, a new approach to biofabrication of tissue constructs based on a magnetic trap has been implemented [1]. Thus, the physical scaffold is replaced by a magnetic field with special structure. The main advantage of the scaffold-free approach is the absence of contact with synthetic biomaterials, therefore, the elimination of the possible reaction of cells to foreign bodies. Also, the resulting construct is essentially three-dimensional.

In order to construct a tissue construct of maximum cellular density, tissue spheroids are used as the building blocks of bioengineered constructions [3]. They are small spherical pieces of tissue, consisting of 2000-3000 cells. It is convenient to work with tissue spheroids due to their observable size (~ 0.2 mm), three-dimensional spherical structure and their ability to merge, which is an essential feature of any living tissue.

As an alternative to magnetic fields, an acoustic (ultrasound) field may be utilized for tissue spheroids levitation. Due to the strong dependence of an acoustic field on the shape and dimension of an ultrasound source, its operating frequency, and boundary conditions, it is possible to create complex 3D acoustic traps. When tissue spheroids are placed in a volume exposed to the ultrasonic field, a so called "acoustic radiation force" arises, which is a result of momentum transfer from the acoustic wave to absorbing or scattering objects. Amplitude and direction of the radiation force depends of the specific field structure. For instance, if an acoustic field in the form of a standing wave is used, and the wavelength is greater than spheroids diameter, then the resulting radiation force moves the spheroids to nodes of acoustic pressure.

New features come with a combination of magnetic and acoustic fields. The magnetic field is convenient for holding a large volume of spheroids, but has limited capabilities in the formation of fine structure. An ultrasound field, in contrast, is more suitable for the formation of small traps. Thus, the combination of magnetic and acoustic fields allows forming of complex three-dimensional structures directly in a nutrient medium. This approach was used in our work. A ring was chosen as the first tissue structure to be formed, which was supposed to be the first step in creating tubular structures for the biofabrication of blood vessels.

2.METHODS

The main goal of the current study was to obtain a quick levitation assembly of a construct from tissue spheroids randomly distributed in the working volume of the liquid nutrient medium in combination with a non-uniform magnetic field and a standing ultrasound field. The construct formed in the volume of such a magneto-acoustic trap appeared as a result of a combined action of gravitational, magnetic and acoustic forces. In this case, the gravitational forces are compensated by magnetic forces in the vertical direction, and due to the magnetic gradient in the horizontal plane, tissue spheroids move towards each other, being levitated above the bottom of a test-tube.

By applying acoustic radiation force, it is possible to change the shape of the structure. The resulting construct may have spherical, toroidal, ellipsoidal, or other shape, which is determined by the specific choice of magnetic and acoustic field configurations. To form a network of branching channels, it is necessary to apply an acoustic field in addition to the magnetic field. The desirable acoustic field is a result of a specially selected combination of standing and propagating ultrasonic waves generated by one or more radiators. As a result, a trap may form a tissue construct of chosen structure under the action of constant acoustic radiation forces. This approach allows us to create one or more branching channels in the construct, and their sizes and shapes are determined by the number of acoustic transducers and their spatial arrangement. The channel should be designed in advance using three-dimensional numerical modeling of the acoustic field, which results in values of amplitudes, frequencies and phase shifts for each specific acoustic transducer located in a certain place outside the fusion region. The idea of vascularized tissue consists of two steps: forming assembly of the construct on the first step with the subsequent formation of internal structures. Channel formation occurs when the acoustic radiation force acts on the tissue spheroid greater than the vector sum of other forces acting on the spheroid in the given area. Magnetic force tends to pack tissue spheroids as closely as possible, in turn, the acoustic radiation force affects tissue spheroids in certain areas of the construct and thereby expands the spheroids and creates channels. If the magnetic force is greater than the acoustic radiation force, the formation of channels will not occur. Due to this method, biofabrication of a tissue-engineering construct occurs in a few seconds with a network of branching channels with a diameter ranged approximately from 300 μm to 900 μm, depending on ultrasonic field frequency and intensity. After the fabrication stage, the levitating construct remains under the influence of the trapping fields until the process of tissue spheroids fusion occurs.

To create a ring-shaped tissue construct in the current study, the experimental setup consisted of two ring-shaped magnets connected to each other by opposite poles (Fig. 1a,b). A cylindrical notch was made in the center of the setup to accommodate a cylindrical piezoelectric transducer. Magnetic part of the experimental setup creates a local minimum of the magnetic field potential. Due to the inhomogeneity of the magnetic field and the fact that the relative permeability of the spheroids differs from that of the background fluid, a magnetophoretic force appears. It induces particles movement toward regions with low potential of the magnetic field.

The acoustic transducer in a form of a piezoelectric cylinder with an inner radius of R_{in} =16 mm, outer radius 22 mm, and a height of 20 mm was used. The material of the transducer was the PZT-4 piezoceramics. The working zone of the assembly was located inside the piezoelectric transducer, where a cylindrical standing acoustic wave was excited, and, in turn, an acoustic radiation force appeared. It acted from antinodes to nodes and caused formation of tubular structures from tissue spheroids, like rings or tubes. The frequency of ultrasound wave f was chosen under the two following conditions: it had to be close to resonance for both the transducer thickness, and the standing cylindrical wave inside the piezoceramic tube.

To predict the result of construct assembly, numerical modeling was performed. Simulation of a three-dimensional inhomogeneous static magnetic field in a paramagnetic medium from two permanent magnets was performed using the finite element method. The ultrasound field was calculated in accordance with the found field of displacements, created by the piezoelectric transducer, using the Multiphysics computational program COMSOL. Then a particle trajectories equation was solved under action of these fields. During this calculation, the following forces were considered: a magnetophoretic force based on the difference between medium and particle magnetic permeabilities, an acoustic radiation force, appeared after wave scattering on tissue spheroids, a drag force affecting the time of assembly, an elastic force of particle-particle interaction, and a gravity force. Physical characteristics of the simulated particles were chosen in accordance with experimental measurements [3]. To calculate the acoustic radiation force, the Gor'kov's potential was used [4]. This approach is a fairly good approximation for the case when the wavelength is much larger than the scatterer size [5].

3.RESULTS AND DISCUSSION

Both in the computer simulation and experiments, tissue spheroids were collected in the nodes of a standing ultrasound field, levitating in a nutrient medium. Numerical simulation showed that the strongest acoustic radiation force acts in the center of the piezoelectric transducer, due to high gradients of the

Gor'kov's potential in this region. This fact means that the diameter of the resulting tissue ring depends on the radius of the first node (Fig. 1c). The spatial structure of the standing acoustic field inside the cylinder is described by Bessel function $J_0(kr)$, thereby the node of the field can be found from the relation $J_0(kr_i) = 0$. The first node corresponds to the condition $r_1/\lambda \cong 0.3827$. Because the transducer frequency was $f = 780$ kHz and the speed of sound at 37 ℃ was approximately 1530 m/s, the radius of the assembled ring had to be $R \cong 0.74$ mm, which led to the diameter of the tissue ring being around 1.5 mm. The same sizes of spheroids constructs were obtained in both the experiment and numerical simulation. Varying the frequency of the acoustic wave, we could change the diameter of the construct. In addition, if the amount of tissue spheroids and the working volume were sufficiently large, the formation of the second ring was possible, which corresponded to the second node of the standing wave (Fig. 1d). It is important to note that such ultrasound field had quite a low intensity and thus did not damage spheroids. Maintaining configuration of the ring for 18 hours allows spheroids to fuse (Fig. 1 e,f) – this indicated that the spheroids remained viable.

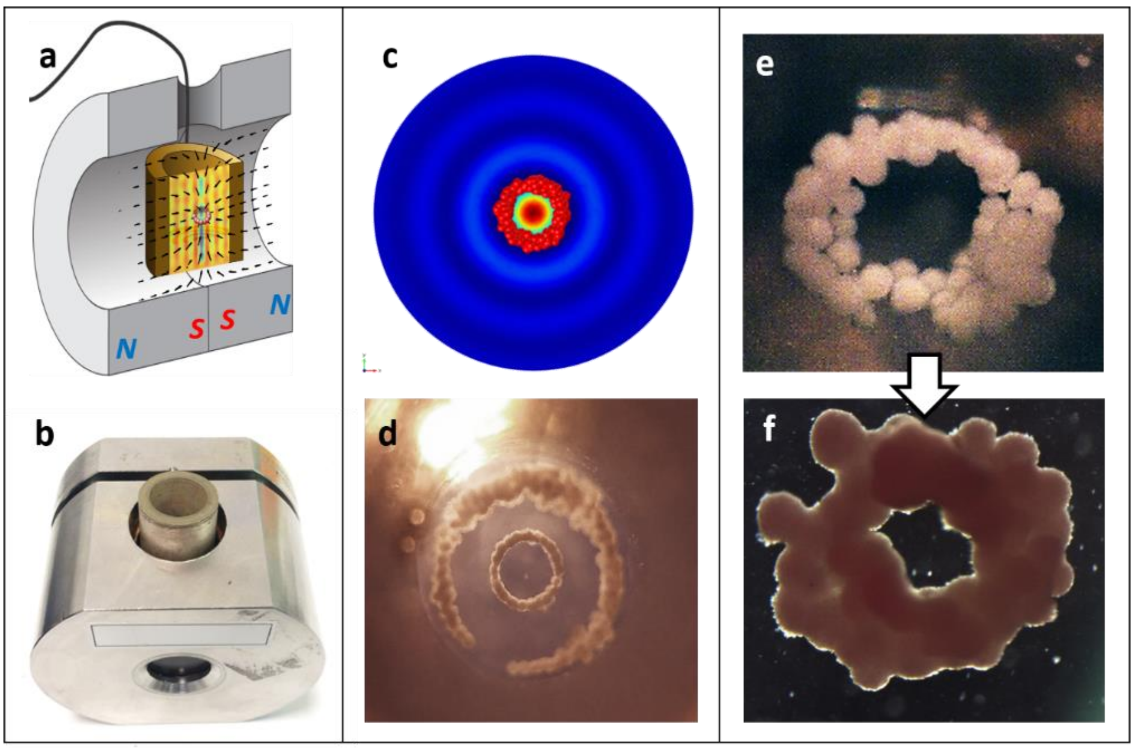

Figure 1: (a) A design and (b) a picture of the magneto-acoustic setup with the cylindrical ultrasonic transducer. Power lines show the direction of the magnetophoretic forces, the area inside the piezoelectric transducer shows the distribution of acoustic pressure amplitude; (c) results of numerical simulation of a spheroids assembly in the magneto-acoustic field. Background color shows the amplitude of Gor'kov's potential; (d) experimental distribution of tissue spheroids in the magneto-acoustic field, formation of the second ring. The diameter of the inner ring is 1.5 mm.; (e, f) fusion of the tissue spheroids into a ring in 18 hours. The diameter of the ring is 1.5 mm.

4. CONCLUSION

The tissue spheroids were assembled using the combination of acoustic and magnetic fields in a state of levitation directly in liquid nutrient medium. The assembly of tissue construct, and sequential tissue spheroids fusion agreed well with developed predictive mathematical models and computer simulations. The proposed approach opens new horizons in the use of magnetic and acoustic fields for the scaffold-free biofabrication of tissue constructs of various forms and functionalities.

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