Levitation of Tissue Spheroids and Spheroid-Mimicking Plastic Beads in a Liquid Using Ultrasonic Fields of Complex Structure for Scaffold-Free Bioprinting

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Statement of Purpose: Use of tissue spheroids as building blocks is a promising approach in organ printing technology [1]. Several physical principles could be used for manipulating the spheroids and forming desired tissue structures. One possible approach used here is trapping and transporting spheroids by an ultrasound wave. The use of the effect of acoustic radiation force for cell manipulation has shown a rapid development in recent years. For example, it has been demonstrated that biofabrication can be performed using surface acoustic waves in two and three dimensions [2, 3]. Another approach is to use a standing bulk wave that is formed when an acoustic resonance is excited in a fluid-filled chamber [4]. We have reported a study where acoustic levitation of living tissue spheroids was performed using standing waves [5]. In this work we present further results of such an approach aimed toward the development of a scaffold-free bioprinting.

Methods: In order to levitate small particles an experimental set-up was built that generates an ultrasonic standing wave in a liquid. The ultrasonic source was made from piezoceramics and had frequency of about 1 MHz that could be tuned within some range to achieve a resonance. The set-up was used to levitate either spheroids or, to develop various acoustic regimes, spheroid-mimicking polystyrene beads. Tissue spheroids were biofabricated from living primary sheep chondrocytes and NIH 3T3 murine line cells using micromolded non-adhesive agarose hydrogel [6]. The suspension of tissue spheroids or plastic beads were immersed in the DMEM culture medium which was insonified by an acoustic standing wave. The levitation was monitored using a monochrome video camera (Fig.1). Theoretical analysis of the ability of the designed set-up to levitate spheroids was performed based on the previously developed theory that allows radiation force calculation in the acoustic field of arbitrary structure [7].

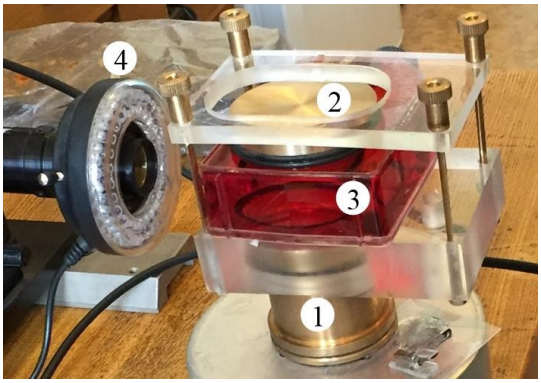

Figure 1. Experimental set-up for ultrasonic levitation of tissue spheroids. 1 – ultrasound source, 2 – reflector, 3 – culture medium, 4 – video camera.

Results: Experiments with the spheroid-mimicking polystyrene beads have shown that ultrasound levitation can be effectively performed when the acoustic pressure amplitude does not exceed 0.1 MPa, which is well below cavitation threshold in water. This result indicates that ultrasonic levitation can be performed without creating a mechanical damage to tissue spheroids. Further histological analysis of tissue spheroids confirmed the integrity and viability of 3D structures after ultrasonic action. Levitation of NIH/3T3 tissue spheroids was achieved at the same pressure levels. The standing wave forced the beads or spheroids to form periodic structures that consisted of the chains of particles (Fig. 2). Theoretical predictions of the acoustic radiation force that corresponds to the levitation threshold were in a good agreement with the observations.

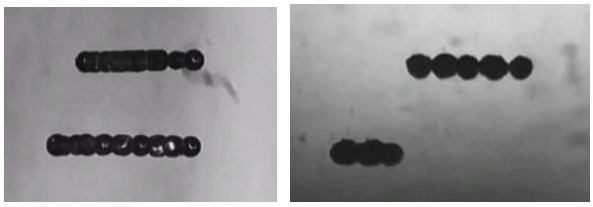

Figure 2. Levitation of chains formed from 175-μm diameter polystyrene beads (left) and NIH/3T3 tissue spheroids (right) in an ultrasonic standing wave.

Conclusions: Ultrasound provides a unique possibility to levitate tissue spheroids and to form chain-like or more sophisticated structures in a culture medium. The results obtained in this study demonstrate that ultrasound can be effectively used to manipulate tissue spheroids and thus potentially can aid the development of a scaffold-free bioprinting of different tissue structures.

Part of study performed at Moscow State University was supported by RFBR 17-02-00261.

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