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Proceeding Paper

Lymph Node-on-Chip: Possibility of Passage of Polymer Capsules to Metastases Model †

Anatolii A. Abalymov [1,2,*], Sergei V. German [1,3], Maxim A. Kurochkin [1], Yuliya Kan [1], Dmitry A. Gorin [1] and Marina V. Novoselova [1]

[1] Skolkovo Institute of Science and Technology, Bolshoy Boulevard 30, bld. 1, 121205 Moscow, Russia; email1@email.com (S.V.G.); email2@email.com (M.A.K.); email3@email.com (Y.K.); email4@email.com (D.A.G.); email5@email.com (M.V.N.)
[2] Science Medical Center, Saratov State University, Astrakhanskaya 83, 410012 Saratov, Russia
[3] Institute of Spectroscopy of the Russian Academy of Sciences, Fizicheskaya, 5, 108840 Moscow, Russia
* Correspondence: anatolii.abalymov@sgu.ru
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Abstract: We developed a lymph node-on-chip (LNOC) that mimics a secondary tumor in lymph nodes for drug testing. The LNOC has a collagen sponge with 4T1 cells inside, resembling the morphology of a human LN. With LNOC, we were able to evaluate the effect of polymeric microcapsule size on penetration in a secondary tumor model. Our study found that 0.3 μm capsules were the most effective for delivery, demonstrating the LNOC's potential for particle and contrast agent testing. The LNOC is an alternative for analyzing carriers for drug delivery and different contrast agents for cells, tissues and organs.

Keywords: lymph node-on-chip; cellular spheroid; polymer nanocapsules

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1. Introduction

Nowadays, 3D platforms for testing drugs, contrast agents, nano- and microparticles in vitro are being actively developed to improve their efficacy [1,2]. These bioengineering solutions allow for the system to be tested under real-world conditions without the need for animal testing [3]. By applying engineering methods, the resulting structures can be fine-tuned for installation and long-term observation in imaging systems [4].

As a part of our project, we have designed a lymph node-on-chip (LNOC) using microfluidics. The LNOC is a tissue-engineered model that imitates the formation of a secondary tumor in the lymph node due to the metastasis process (Figure 1a). The chip comprises a collagen sponge, resembling the morphology and porosity of a natural human lymph node, which holds a 3D spheroid of 4T1 cells, mimicking a secondary tumor in the lymphoid tissue. We conducted an experiment to analyze the impact of the size of drug carriers or contrast agents on the penetration and accumulation of particles in 3D spheroids that simulate secondary tumors. To achieve this, we mixed bovine serum albumin (BSA)/tannic acid (TA) capsules of 0.3, 0.5, and 4 μm with lymphocytes and passed them through the chip. Our results indicate that the 0.3 μm capsules were the most effective for delivery as they could penetrate the spheroid well. This research highlights the potential of our LNOC for evaluating particles and contrast agents, and its capability to analyze and comprehend the efficiency of drug delivery vehicles and contrast agents in organs and cells. Additionally, the outcomes of this study can be utilized to create other types of lab-on-chip systems.

2. Results and Discussion

The objective of chip development was to create a collagen extracellular matrix (ECM) model using a collagen sponge with similar pore size (Figure 1b). Microfluidic devices made of biocompatible materials were used to mimic the functional characteristics of the native lymph node (LN), such as the collagen ECM structure and lymphocyte flow. The chip has a main channel for the culture medium to flow through, with the growing cell medium supplied and extracted through stainless steel and silicon tubes. A vacuum pump is used to press the chip body against the glass for microscopy. The collagen sponge in the center of the chip has interconnected open porosity that mimics the collagen ECM of the LN. The spheroids used in the chip are 3D human tumor spheroids, chosen for their ability to reflect the tumor microenvironment. The chip allows for the observation of how drugs and carriers pass inside the spheroid to the desired depth, which is crucial as spheroids have three zones: necrotic core, static, and proliferative zone, with varying thicknesses depending on the type of cell.

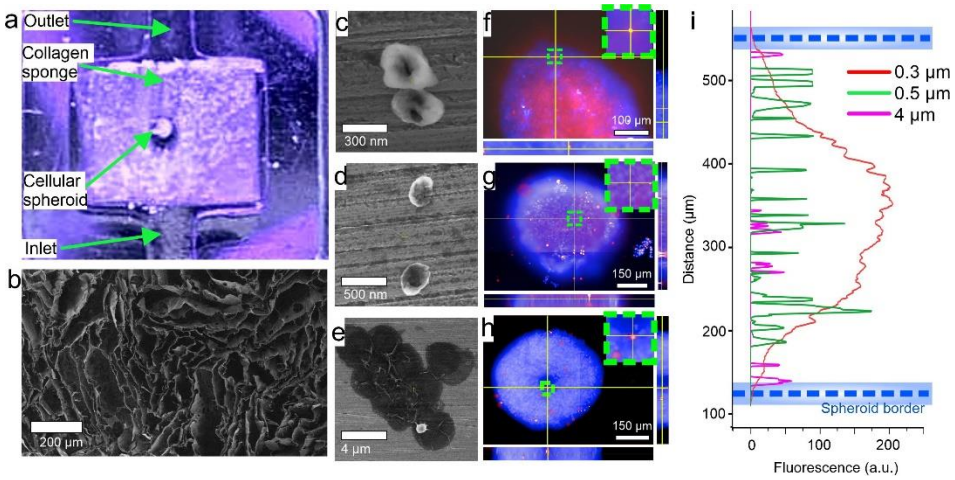

Figure 1. (**a**) Macrophotograph of obtained LNOC. (**b**) SEM image of collagen sponge. (**c**–**e**) SEM images of polymer capsules of (**c**) 0.3, (**d**) 0.5 and (**e**) 4 μm diameter. (**f**–**h**) Orthogonal fluorescence images of cell spheroids with internalized (**f**) 0.3, (**g**) 0.5 and (**h**) 4 μm capsules. (**i**) The fluorescence brightness profile along the dashed yellow line in (**f**–**h**), which shows the fluorescence distribution of capsules 0.3 (red), 0.5 (green), and 3.5 (purple) in the cell spheroid, the borders of which are marked with blue.

The objective of this study was to investigate the potential of a developed system for evaluating the ability of drug delivery systems/contrast agents of different sizes to penetrate secondary tumors in the lymph node (LN). The use of nanoparticle systems as contrast agents for detecting the sentinel lymph node (SLN) and delivering anti-cancer drugs to tumors is of great interest, and the researchers sought to investigate the impact of polymer particle size based on BSA/TA on tumor cell penetration and absorption using the developed LNOC. We utilized BSA-Cy5/TA particles with sizes of 0.3, 0.5, and 4 μm, which were obtained using the layer-by-layer coating method on vaterite particles (Figure 1c–e). To model a secondary tumor, a 4T1 breast cancer spheroid was created using an ultra non-adhesive plastic surface and placed in the center of the chip well. To image the cells and capsules inside the LNOC, dyes such as Calcein Am (for lymphocytes), Hoechst (for spheroid cell nuclei), and Cy5 (for particles) were used. T lymphocytes were used to simulate the lymph flow and a mixture of T-cells and capsules (0.3, 0.5, or 4 μm) and fluids were injected into the microfluidic system using a syringe pump. The volume flow rate through the LNOC was 0.65 mL/h. The presence of lymphocyte cells and polymer capsules in the sponge surrounding the cell spheroids was evaluated, and it was found that the capsules and cells successfully passed through the sponge surrounding the spheroid.

Using confocal microscopy (as shown in Figure 1f–h), we assessed how efficient capsules pass through a collagen sponge. The fluorescence micrographs displayed that both lymphocyte cells and capsules were able to successfully move through the sponge that surrounded the spheroid. However, there were slight modifications in the spheroid's shape due to shear stress. Regardless of size, fluorescence microphotographs of the sponge with the spheroid showed that capsule passage was successful, with a large number of 0.3 μm capsules entering the spheroid. Depending on factors such as the density of the intercellular matrix and medium flow around the spheroid, larger objects can fall into the intercellular space, which can range from 20 to 500 μm. Most 4 μm particles accumulated on the surface of the spheroid, while 0.5 μm particles were found at a depth of ≈75 μm, and 0.3 μm particles penetrated the cellular spheroid the deepest (≈125–150 μm from the surface) (Figure 1i).

3. Experimental Section

3.1. Materials

Chemicals and solutions used were purchased from Sigma-Aldrich and Gibco. Collagen was purchased from BioProduct Ltd., Moscow, Russia. DI water was used for all solutions. Polydimethylsiloxane elastomer was purchased from Dow Chemical, Midland, MI, USA (Sylgard 184).

3.2. Preparation of $CaCO_3$ Particles

Vaterite particles 4 μm in diameter templates were synthesized by mixing $CaCl_2$ and Na_2CO_3 solutions under stirring. Vaterite particles 0.3 and 0.5 μm were prepared by mixing $CaCl_2$ and $NaHCO_3$ solutions in ethylene glycol.

3.3. Capsules Preparation

BSA-Cy5 was loaded onto $CaCO_3$ cores using FIL technique, followed by centrifugation and washing. Capsule shells were made of BSA-TA bi-layers. Cores were dissolved with EDTA or HCl to create polymeric capsules, which were purified and washed with DI water.

3.4. Microfluidic Chip Fabrication

To fabricate the microfluidic chip, we used silicone elastomer Syl-gard 184, and the channel was covered with glass. Silicone tubes were attached to the chip body through stainless steel tubes. To create the master mold for casting the chip body, we utilized photopolymer 3D printing with water washable resin Elegoo (Elegoo Inc., Shenzhen, China). To prevent inhibition of the elastomer polymerization during the casting of the silicone elastomer, a separating layer was employed.

3.5. Formation of the Collagen Sponge—Model of Lymphoid Tissue

A collagen sponge was made using the freeze-casting method by placing 2 wt. % collagen gel in a mold with directional crystallization provided by cooling the metal substrate with liquid nitrogen. After freeze-drying, the sponge was stabilized using EDC/NHS crosslinking. The resulting porous material was sliced into thin pieces for use in a lymph node-on-chip. Pore size was adjusted by changing the temperature of the cooled mold wall, and pieces with long interconnected pores were obtained by cutting from the steady-state growth zone during freeze-casting.

3.6. Cell Cultivation:

4T1 and Jurkat cells were cultured in DMEM and RPMI 1640 supplemented with 10% FBS and 100 μg/mL penicillin/streptomycin. The media were replaced every 3 days, and the cells were maintained in a humidified incubator at 5% CO_2 and 37 °C.

3.7. Formation of Tissue Spheroids:

Tissue spheroids were formed from 4T1 cells using ultra-low adhesion Corning spheroid microplates according to the manufacturer protocol. The cells were incubated for 3 days.

3.8. Fluorescence Microscopy:

Jurkat cells were stained with Calcein Am and 4T1 spheroids were stained with Hoechst 33342 for 15 min. Z-stacks were taken and experiments were evaluated using Imagej software.

4. Conclusions

We developed a microfluidic lymph node-on-a-chip (LNOC) that can mimic the lymph node with secondary tumor in three dimentional space. To assess the internalization of polymer capsules into metastasizing cells, we employed the LNOC. We simulated lymphoid tissue in the chip using a collagen sponge and spheroids based on 4T1 breast cancer cells. Capsules were mixed with lymphocytes and injected at a flow rate that matched the lymph flow, and we discovered that 0.3 μm capsules were the most efficient for delivery. These findings demonstrate the potential of the LNOC for particle and contrast agent testing, as well as its attractiveness as an alternative for drug delivery vehicle and contrast agent analysis.

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Conflicts of Interest: The authors declare no conflict of interest.

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