Simultaneous spheroid formation and nanoparticle encapsulation by acoustic microstreams

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Introduction

3D cell spheroids are attractive constructs with numerous applications in biomedical research, from tissue engineering to drug screening. Recently, we reported an acoustic platform with the ability to rapid production of spheroids as fast as 10 s.[1] In this method, cells are trapped in a controllable microstream which is generated by an acoustic source and are adhered together with type I collagen which is a natural ECM. Since the acoustic spheroid formation relies on the physical agglomeration of particles, inorganic particles can also be encapsulated in the spheroids.

Objective

In this study, we exploited the acoustic spheroid formation to encapsulate nanoparticles in the spheroids too. Poly β-amino ester (PBAE):pDNA complex nanoparticles, which are widely used for introducing genes to cells[2] , are used as a proof of concept to show the potential of the method for simultaneous spheroid formation and gene delivery.

Method

Device fabrication and setup: The mold was produced by standard photolithography of negative photoresist (SU-8 2050) on a silicon wafer. For replication, PDMS was poured on the master mold treated with silane and was incubated at 70 degrees Celsius overnight. After liftoff, the channel was bonded to a glass substrate by oxygen plasma surface treatment. A piezoelectric STEMiNC (SMBA4510T05M) was attached next to the channel and was controlled by a function generator (AFG3011C, Tektronix, USA).

Cell culture: MCF-7 were cultured in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% FBS and 1% penicillin/streptomycin.

Cell-Collagen preparation: 8 volumes of type I atelocollagen solution, 3 mg/ml (PureCole) were gently added to one volume of 10X culture media and the solution was neutralized by 0.1 M NaOH. The solution was diluted in serum-free media and Methylcellulose (MC) was added to reach the concentration of 0.42 mg/ml Collagen+ 0.4 w/v MC.

Nanoparticle synthesis:

Cy5 labelled pDNA and PBAEs were diluted in 25 mM sodium acetate buffer (pH = 5.2 ± 0.2) to reach the concentration of 60 µg/mL for pDNA and 1.8 mg/mL for PBAE. 30 w/w, one part of PBAE solution was added to 1 part of pDNA solution to reach 30 w/w nanoparticles, and vortexed for 10 s, and incubated for 10 min.

Spheroid formation and nanoparticles encapsulation mechanism

Upon activation of the acoustic platform via radio frequency signals, the bubbles and sharp-edges embedded in the microfluidic channel oscillate and create strong boundary-driven acoustic streaming. The hydrodynamic forces induced by acoustic microstreams can trap and enrich the cells in the vortex eye. Figure 1 represents the schematic of cells and nanoparticles trapping process and enrichment in the microfluidic platform.

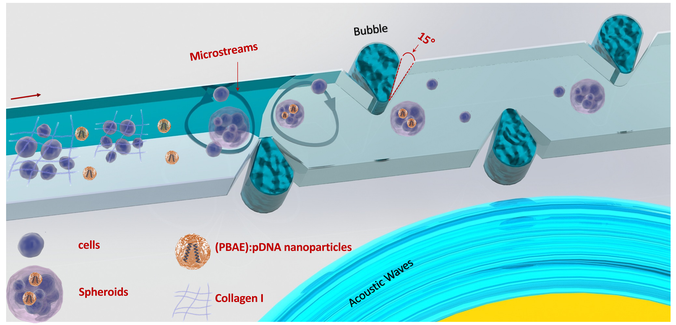

Figure 1. Acoustic Microfluidic platform for simultaneous spheroid formation and nanoparticle encapsulation.

Results

As the suspended particles such as cells and nanoparticles are exposed to acoustic microstream, the drag forces from the vortex trap the particles. The presence of collagen I allow for rapid attachment of cells to form coherent spheroids. Moreover, the drag forces from the microstream can also enrich nanoparticles in the vortex, which leads to a higher concentration of nanoparticles in the spheroids. Figure 2 a shows the cell-nanoparticles spheroids formed in the microfluidic channel using acoustic waves. To visualize and quantify the nanoparticle enrichment and encapsulation in the spheroids, fluorescently labeled plasmid were complexed with PBAE polymer to form nanoparticles. Figures 2 b,c,d show a spheroid that was retrieved and incubated overnight. The cyan color illustrates the nuclei stained with Hoechst 33342 while the red is representative of the (PBAE):pDNA complex nanoparticles which are labeled by Cy5. As it can be seen, the spheroid shows a compact structure after 24 hours, and the nanoparticles are homogenously distributed throughout the spheroid.

The fluorescent analysis by Image J showed the Cy-5 fluorescent intensity in spheroids was 51.783±18.402 while the number outside the vortex was 15.633±7.181, showing approximately 3.2 times increase in the concentration.

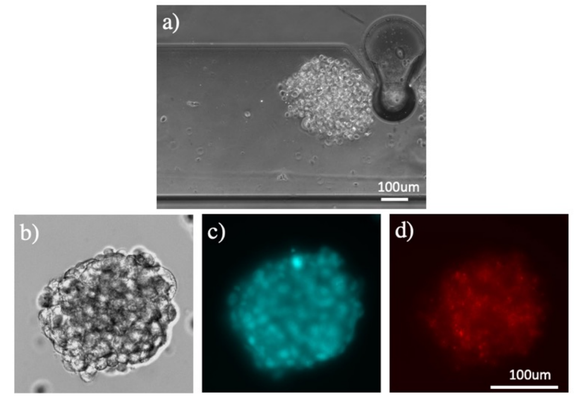

Figure 2.a) Acoustic trapping of cell and. b) An acoustically formed spheroid/NP composite c) cell nuclei stained by is Hoechst 33342 d) nanoparticles.

Conclusion

In this study, we report on the rapid formation of spheroids and the simultaneous addition of concentrated nanoparticles throughout the spheroids which lay the groundwork for efficient gene delivery into the spheroids. The system showed not only the ability to homogeneously encapsulate nanoparticles in the spheroids, but also more than a 3-fold increase in the nanoparticles concentration in the spheroids by trapping them in the vortex.

Acknowledgement & References

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