HUMAN STEM CELL-DERIVED TISSUE ORGANOIDS AS A POTENTIAL TOOL FOR DRUG DELIVERY TESTING

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Abstract

Advancement in organoid cultures from human pluripotent stem cells allows studying complexity of tissue specific interactions *in vitro*. This organ in a chip approach provides an important tool for translational research. Successful development of nanoparticles and nanostructures for clinical use has increased the need for more reliable and high-throughput experimental models beyond conventional cell culture. Human induced pluripotent stem cells (iPSCs) can be differentiated to multiple cell types including tissue-like organoids. Here, we study the interaction of nanoparticles with differentiated tissue cells and analyse their effects on various organoids *in vitro*. Human tissue organoids are differentiated from iPSCs by growth factor and by manipulation of various developmental signal pathways with inhibitors. The 3-dimentional structures of the bodies, with structural features similar to the appropriate human organ/tissue are obtained by embedding in a matrix. In order to test organoid as a model for interaction with nanoparticles, the organoids are injected with nano-sized particulate triggers such as glucans. The various tissue models are compared for their nanoparticle-induced stimulation by screening for inflammatory cytokines. Described model will allow studying human tissue interaction with nanoparticles, which for example are used in drug delivery.

Keywords: Nanoparticles, organ in a chip, lung-organoid, stem cells, lipopolysaccharide

1. INTRODUCTION

During development, the human lungs arise from the anterior foregut of the mesoderm as tubulated structures, giving rise to predominantly pulmonary epithelium and associated tissues such as basal cells and mesenchyme. Recent progress in the regenerative medicine, as well as defining stem cell niche factors that are important for lung development, led to the establishment of an in vitro three-dimensional (3D) culture system, termed pulmonary (lung) organoids [1]. This model system is useful in a number of applications ranging from *in vivo* inhalation toxicology study for smokers and harsh-environment workers to multiple basic research approaches [1,2]. The main advantages of the organoid model comparing to conventional cell cultures are the complexity of different cell types and specific tissue like microenvironment. The tool also provides a high-throughput platform for study of the differentiated human airway like tissue. The use of patient derived lung organoid models for drug screening is one of the most futuristic methodology of personalized medicine [3].

The alveolar membrane is the largest surface of the body in contact with the outside environment. Therefore lungs are in the front line of host defence, furthermore healthy lung are infiltrated by number of immune cells of different phenotypes [4]. The immune cells together with lung epithelia are initiators of innate immune response, as they produce numerous cytokines directly in response to injury or pathogens [5]. Majority of these studies are conducted in mice models lung cancer cell lines, which has a limitation of being too distant from human lung tissue physiology. Innate immune triggers such as LPS-induced lung models have been used extensively to explore mechanisms of multiple diseases and provide useful information on the discovery of novel biomarkers and drug targets. Here, we are using an induced pluripotent stem cell derived 3D lung organoid system as the study model. This model system in combination with physiologically-inert nanoparticles

are used to establish the interaction of nanoparticles within the pulmonary cells and also to check how lung tissue can generate inflammatory response in an immune less environment.

2. MATERIALS & METHODS

Undifferentiated human induced pluripotent stem cells were maintained in mTESR-1 (Stemcell Technologies) media on hES-qualified matrigel coated tissue culture plates, according to the manufacturer's instructions.

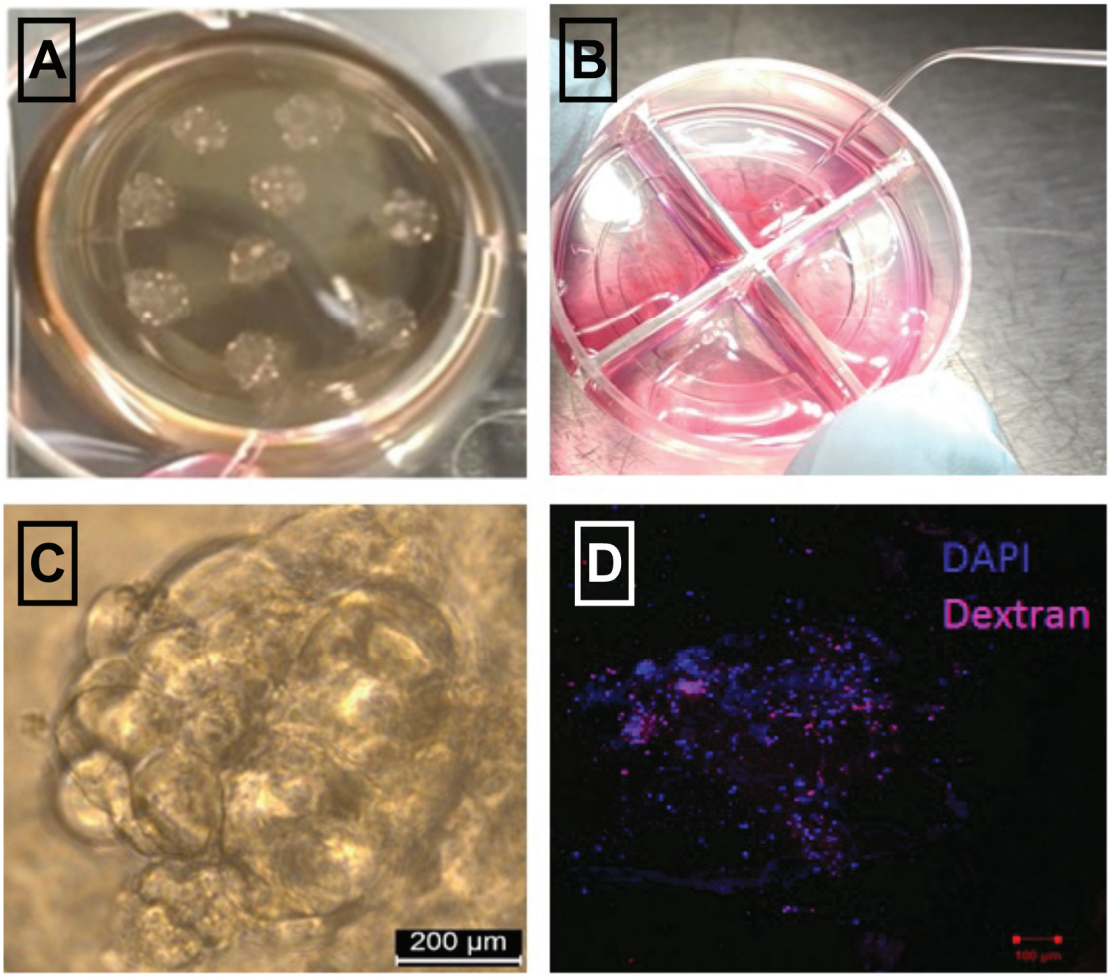

Figure 1 Experimental setup of lung organoid (LO) treatment with nanoparticles. A - Los embedded in matrigel to form 3D structure. B - Microinjection of LO's with Dextran nanoparticles. C - Brightfield microscopic image of budding LO. D - Visualization of dextran nanoparticles labelled with TRITC inside the LOs

The iPSCs are differentiated into 3D-lung organoids by a three step differentiation process (as described in De Luca, *et al* [5]) which is an adaptation of Dye BR *et al* [6]. iPSCs on reaching ~80% confluency are fed with endoderm differentiation medium (RPMI1640 + P/S (500U/ml) + 100ng/ml Activin A and defined FBS ranging 0% - 0.2% - 2% over 3 days) to develop definitive endoderm. The endodermal layer is fed with foregut differentiation media daily (Advanced DMEM/F12 + N2 supplement+ B27 supplement + HEPES (15mM) + Glutamine(2.5mM) + P/S (1%) + Noggin (200ng/ml) + SB431542 (10uM)) for 4-5 days. After ~3-4 days of exposure to foregut differentiation medium, 3D structures will start to become visible and by day 5 is possible to see free-floating anterior foregut spheroids that have detached from the dish. Collect spheroids from every well under a microscope using a 200-µl pipette tip cut to make wider and pool ~30 spheroids into a 1.5-ml

microcentrifuge tube with 25ul media. Mix the media-spheroid mixture with 1:1 volume of matrigel (i.e 25 ul) and place like a bead over Nunc delta surface plates and is fed with LO media weekly (foregut media + FGF4 (500ng/ml) + CHIR99021 (2uM) + 1% FBS). Around 15-20 days 3D-organoids which exhibit features of human lung tissues are obtained, which can be split and maintained (**Figure 1 A, C**).

Labelled dextrans are hydrophilic polysaccharides most commonly used in microscopy studies to monitor cell division, track the movement of live cells, and to report the hydrodynamic properties of the cytoplasmic matrix. The labelled dextran is available at different molecular weights based on the particle size. Here we are using a 3000 MW, neutral dextran which has particulate size at nano-level and are labelled with Tetramethylrhodamine (TRITC). Dextran particles are dispersed in PBS (solubility <50 mg/mL for 3000 MW dextrans) correspondingly to maintain particularity of the nanoparticle. The dispersed dextran nanoparticles at estimated 10 particle per cell concentration was injected into the lung organoids using a Sutter instruments microinjector under a stereo microscope (**Figure 1 B**).

The lung organoids after 24hrs of dextran microinjection are collected and imaged under microscope for the nanoparticle to cell interaction (**Figure 1 D**). The organoids are further screened for inflammatory responses - production of cytokines. These readouts are obtained at mRNA level by qPCR using a Roche LightCycler 480 RT-PCR and imaging under Zeiss confocal microscope.

3. RESULTS & DISCUSSION

Lipopolysaccharide (LPS), a major component in gram-negative bacteria, has been used to induce several disease models of lung tissue [2]. Previous studies using mice models have shown that exposure to nanoparticles such as carbon black, which is a mild air-pollutant as well as latex nanotube, which is considered biologically inert, facilitate pulmonary inflammation via both innate and adaptive immunological impairment [7]. Indeed, it has been reported that nanomaterial exposure itself induces lung inflammation in case of titanium dioxide, gold and platinum spheres, and quantum dots [7]. These immunogenic effects make these particles another inflammatory trigger but not suitable candidates for drug delivery or *in vivo* cell tracking. Here, in our experimental approach LPS is able to induce inflammatory response in the lung organoids by 24 hour treatment and is able to stimulate the production of cytokines such as IL-1b, IL-6 and IL-12 (**Figure 2**).

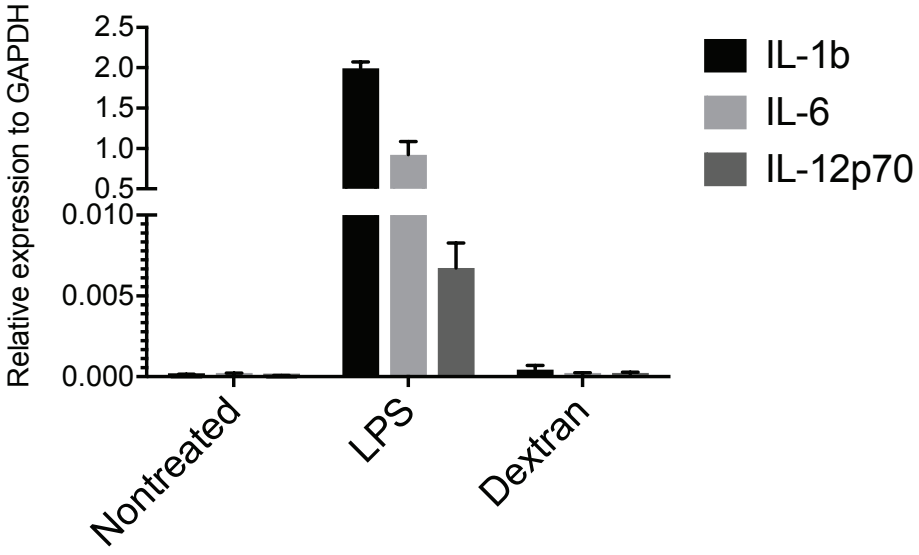

Figure 2 Q-PCR analysis of inflammatory cytokine production by lung organoids (LOs) after microinjection of dextran nanoparticels in comparison with nontreated and LPS treated LOs

But, dextran an inert polysaccharide at nano-size is not able to generate any inflammatory responses in the LOs (**Figure 2**). Here we provide a model where various nanoparticles can be tested for their immunomodulatory properties in tissue like microenvironment *in vitro*. Our results suggest that apart from the particle size, the material of the nanaoparticle could as well play a very important role in its biological activity, and also projects the potential role of dextran nanoparticles in drug delivery. Together with the lung organoid model, these nanoparticles would make an excellent experimental platform for translational medical research.

4. CONCLUSION

Using iPSCs derived lung organoids, we are able to analyse the cytokine response to various triggers including nanoparticles. Here we show that lung like tissue derived from human iPSC is able to produce inflammatory cytokines, IL-1b, IL-6 and IL-12 in response to LPS, while the nanoparticles microinjected into the organoid do not trigger the cytokines expression. After further development this approach may provide *in vitro* platform to test lung cells responses in more complex 3D environment.

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