A 3D printed collagen structure for lung in vitro models

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A 3D printed collagen structure for lung *in vitro* models

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***Abstract*— The design of a system to model the complex structure of human lung was developed through fabrication of 3D printed collagen type I hydrogel. This model can be applied for the investigation and identification of therapeutic strategies for lung cancer.**

Keywords—Lung, 3D bioprinting, collagen

I. Introduction

Lung cancer is the leading cause of cancer death around the world. Non–small cell lung cancer (NSCLC) is the most frequent histologic type and undergoing to surgical resection. Among the different type of NSCLC, the adenocarcinoma is the most common (about 40% of lung cancer) and arises from the epithelial alveolar cells[1]. Alveoli are the terminal functional structure of the lungs in which the gas exchange between air and blood takes place.

Management of lung disease often includes drug therapy, oxygen therapy, radio therapy, surgery, and lung transplantation. Unfortunately, due to lack of donated lungs many patients on transplantation lists will succumb to the disease before transplantation. Furthermore, the development of safe and effective therapies is currently constrained by the lack of a robust preclinical and experimental models that can reproduce the behavior of tissues. Existing animal models have been developed to study the evolution of disease and identify several key mediators in pathology as an alternative to testing on a living whole-body system[2]. Nevertheless, some therapies that showing efficacy in limiting lung pathologies in pre-clinical animal models have failed human clinical trials due to the different airway anatomy and lung cell biology between animal and human models[3]. For these reasons, there is a crucial need for ***in vitro* models** that can quickly and reliably predict drug safety and efficacy in humans during preclinical studies. Two-dimensional (2D) models enable study of cell responses in a controlled setting with the ability to tweak individual cell responses to soluble cues, matrix molecules and matrix mechanics. Nevertheless, 2D cell culture models are unable to mimic the 3D nature as well as the mechanical and architectural behaviour of lung tissue[4]. Therefore, successful **3D models** have been developed to replicate native lung tissue microenvironment with greater accuracy. Successful **3D models** are those which replicate cell-cell and cell-matrix interactions, while mimicking native matrix stiffness and structure[5] (Fig. 1).

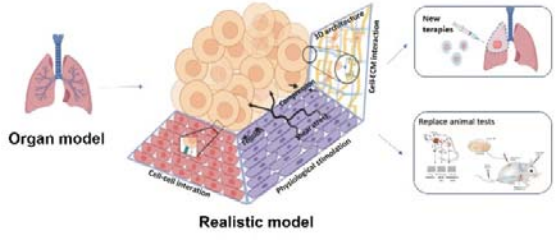

Fig. 1 *Illustration of a realistic model organ model approach.*

Among all the 3D structures, hydrogel systems allowed to mimic human tissue composition for *in vitro* study thanks to their biomimetic features. Hydrogels are water-swollen cross-linked polymeric structures. Depending on the polymeric network and crosslinking mechanism, the hydrogels properties can be tuned. 3D collagen type I hydrogel have been widely fabricated for lung cancer models [6] as it is the most abundant protein in lung tissue. 3-D bioprinting is one of the most promising biofabrication technologies that allowed to design complex 3D structures mimicking living systems. This versatile technique takes computer digital data and reproduces it layer-by-layer by extrusion of continuous filaments or droplets on previously printed successive layers [7]. The principal improvement introduced using this technique is the capability to realize **4D models** by extruding cells laden hydrogels.

Here, we describe the design of a model of alveolar acinus through collagen type I 3D bioprinted structure. Firstly, a optimized collagen hydrogel was fabricated and characterized. Thus, printing parameters were tuned in order to obtain stable structures. Finally, a 3D CAD (computer-aided design) model of alveolar acinus was design according to the real normal size of the human lung acinus with the he future outlook of printing the 3D model in a scaled-up version.

II. Materials and Methods

Collagen type I hydrogel was obtained by dispersing collagen powder into a mixture of DMEM and acid acetic. Two concentration of collagen were tested: 1% wt/v and 2% wt/v coded as COLL1 and COLL2 respectively. The acid pH of solution was neutralized by adding NaOH solution to induce the sol-gel transition. The tube inverting test was used to qualitatively characterized the sol-gel transition of the hydrogel. Preliminary printing tests (ROKIT InVivo, Rokit, Seul) were conducted on collagen pre-hydrogel solution in order to optimize printing parameters and thus to obtain a stable gel structure.

III. Results

The tube inverting test demonstrates that after the incubation of the pre-hydrogel solution at 37°C, the phase transition was observed visually by inverting the bijoux; a gel was defined when no significant flow was observed within 60 s. As shown in Fig. 2, this phenomenon was only observed in COLL2. This result demonstrate that the polymer concentration played a critical role in the gelation behaviour.

The printing of collagen hydrogel was achieved extruding the sol-phase material from the syringe (kept at 4°C to avoid the collagen gelation) into the platform at 37°C in which sol-gel transition occurred. Preliminary tests (Fig. 3) were conducted tuning print parameters in term of fill density (FD), layer height (h), speed flow (v), filament diameter (d), input flow (f) and number of layers (n). As shown in Fig. 4, a square lattice hydrogel pattern was obtained by bioprinting process.

A computational model of a lung acinus was created in CAD (computer-aided design) using the Solidworks® platform. The pulmonary acinus model presented consists in a central respiratory bronchiole surrounded along its surface by spherical units representing single alveoli (Fig. 5).

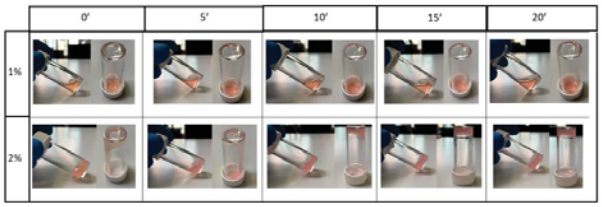

Fig. 2 *Tube inverting test at two different collagen concentration: 1% wt/v and 2% wt/v.*

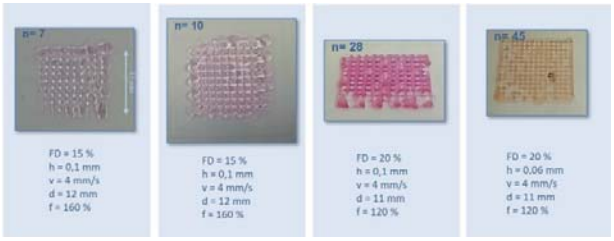

Fig. 3 *Printing test of collagen printed hydrogels.*

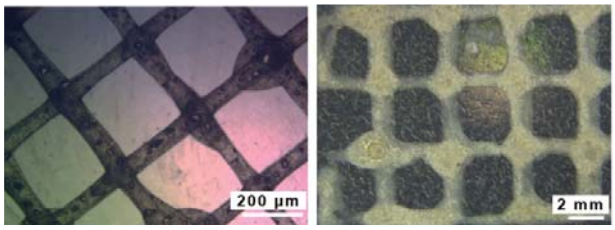

Fig. 4 *Optical microscope images of collagen printed hydrogels.*

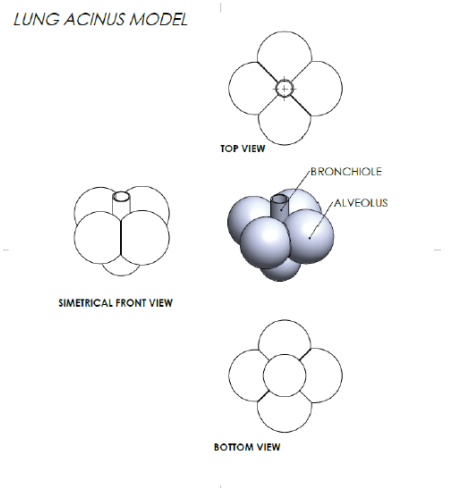

Fig. 5 *3D CAD model of the alveolar acinus. Top, bottom and symmetrical front views.*

IV. Discussion and Conclusions

The obtained results suggest that developed the collagen hydrogels can be applied to produce 3D *in vitro* alveolar models via bioprinting process. Furthermore, a complex 3D model can be fabricated using bioprinting technique starting from 3D CAD model of the alveolar acinus. By adding cells within the collagen hydrogel, a 4D approach will be created boosting the integration of cells into the model.

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