3D in vitro model of the pancreatic acino-ductal unit through additive manufacturing technology

3D *in vitro* model of the pancreatic acino-ductal unit through additive manufacturing technology

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Abstract

This project aims at reproducing the morphology and the composition of the pancreatic acino-ductal unit. More specifically, this work involves the use of additive manufacturing technologies to fabricate a 3D exocrine glandular tissue model that mimics *in vitro* the physiological structure experienced by cells *in vivo*.

Introduction

The pancreatic ductal adenocarcinoma (PDAC) evolves from an intraepithelial neoplasia whose mechanisms of evolution are well known and documented, while the alterations that give rise to the early lesions remain still unclear.[1] These lesions occur within the acino-ductal unit, composed by acinar and ductal cells surrounded by pancreatic stellate cells (PSCs).[2] PSCs strongly influence tumor microenvironment by triggering an intense stromal reaction, which consists in an excessive extracellular matrix (ECM) deposition within the tissue surrounding cancer cells. The stroma plays a key role in tumor progression and limits the drugs perfusion representing a barrier against chemotherapy and radiotherapy.[3,4] Furthermore, the lack of prognosis, the genetic complexity and the tumor heterogeneity make the discovery of new therapeutic options extremely difficult. For this reason, the establishment of an *in vitro* model able to recapitulate the tumoral microenvironment is urgently needed. In line with the 3R principles, the overall purpose of this work is to develop a 3D *in vitro* model of the pancreatic acino-ductal unit which allows to investigate the pathological process of PDAC.

Materials and Methods

The acino-ductal structure was reproduced through a 3D-bioprinting technology (ROKIT InVivo, Rokit, Seul) integrated with an atmospheric pressure plasma jet device (Stylus Plasma Noble, Nadir, Mestre). Specifically, the fabrication process was optimized to achieve high pore interconnectivity, accuracy in glandular geometry and precise control of pore size. To introduce biomimetic cues within the Polycaprolactone (PCL) structure, the plasma surface modification was implemented in a layer-by-layer and automatized manner. Scaffolds were tested to analyze their ability to support human Pancreatic Stellate Cells (PSCs) and Human Pancreatic Ductal Epithelial (HPDE) cell stably expressing activated KRAS (HPDE/KRAS) in mono- and co-culture. The culture system was monitored for 21 days. Additional work was performed to reduce the pore size and improve the model resolution by applying melt electrospinning technology which combines additive manufacturing principles with conventional electrospinning technique.[5]

Results

3D-printed structures morphology was characterized by optical and scanning elec-

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Key words: 3D *in vitro* model; additive manufacturing; PDAC; exocrine glandular tissue model.

Disclosures: Authors have nothing to disclose.

Conference presentation: This paper was presented at the Third Centro 3R Annual Meeting - L'era delle 3R: modelli *in silico, in vitro* e *in vivo* per promuovere la ricerca traslazionale - 30 September - 1 October 2021, Evento online organizzato dal Politecnico di Torino.

Received for publication: 9 July 2021.
Accepted for publication: 7 September 2021.

Biomedical Science and Engineering 2021; 4(s1):194
doi:10.4081/bse.2021.194

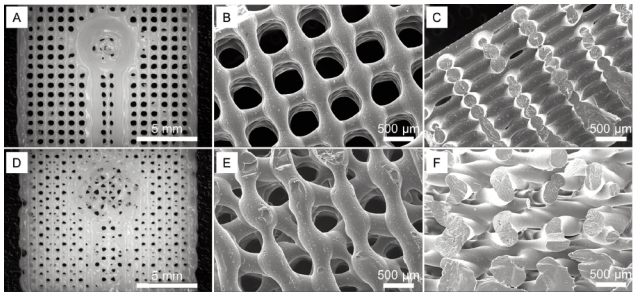

Figure 1. Images from optical microscopy (A,D) and SEM analyses (B,C,E,F). Structures obtained with infill angle of 90° (A,B,C) and 45° (D,E,F).

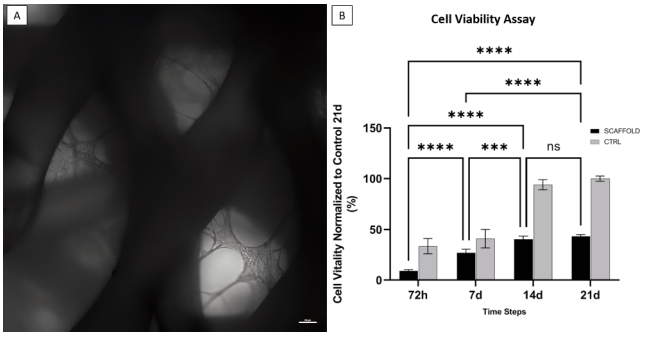

Figure 2. Image from confocal microscopy showing cell-cell interactions within the scaffold pores (A). Cellular metabolic activity throughout 21 days of culture was determined by CellTiter-Blue® Cell Viability Assay (B). Scale bar = 50 µm.

tron microscopy (SEM). The images demonstrated the effectiveness of the optimization process, which led to the formation of a continuous filament, defect-free structures, accurate acino-ductal geometry, and highly interconnected pores. Figure 1 shows that pore interconnectivity significantly improves by varying the infill deposition angle from 90° to 45°. Morphological and cytochemical analysis confirmed the adhesion and proliferation of human stromal cells on the PCL treated scaffolds throughout the entire culture period (Figure 2). Cells were able to colonize the whole 3D structure by forming connections within the highly interconnected pores. Moreover, HPDE/KRAS were successfully cultured inside the acino-ductal geometry.

Discussion and Conclusions

Besides mimicking the physiological human glandular tissue, both in compositional and geometrical aspects, this 3D *in vitro* model could provide a powerful tool to identify new diagnostic biomarkers and establish efficient screening tests. Moreover, it will allow to better study the influence of stroma on the tumor's evolution and perform drug screening.

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