# 4D Printing to fabricate an *in vitro* model of the pancreatic acino ductal unit

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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 a 4D printing system that combines melt electrospinning technology with an automatized layer-by-layer surface functionalization which provides biological cues to the surface of the melt electrospun structure. This 3D exocrine glandular tissue model mimics *in vitro* the physiological structure experienced by cells *in vivo* and serves as a powerful tool to investigate pathological processes such as the Pancreatic Ductal Adenocarcinoma (PDAC).**

***Keywords—4D printing, Melt electrospinning, Pancreatic tumor.***

## I. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a type of exocrine pancreas tumor which currently represents the fourth most common cause of cancer-related death in the European Union with a five-year survival rate of below 9% [1]. PDAC mainly develops in the head of the pancreas, from the progression of pancreatic intraepithelial neoplasia (PanIN) lesions that occur within the acino-ductal unit, composed by acinar and ductal cells surrounded by pancreatic stellate cells (PSCs) (Fig. 1) [2]. Important risk factors can contribute to the progression of PDAC, like smocking, obesity, type 2 diabetes, chronic pancreatitis, and alcoholism [3].

During the tumorigenesis, the PSCs, that are normally located in the periacinar space in a quiescent state, become active and change their morphology in a spindle-shaped, showing a myofibroblasts-like phenotype. Their first activation is determined by an inflammatory stimulus which leads to the recruitment of inflammatory cells into the cancer site (monocytes, T cells, neutrophils, macrophage, and mast cells) [4]. PSCs strongly influence tumor microenvironment by triggering an intense stromal reaction, defined desmoplasia, 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].

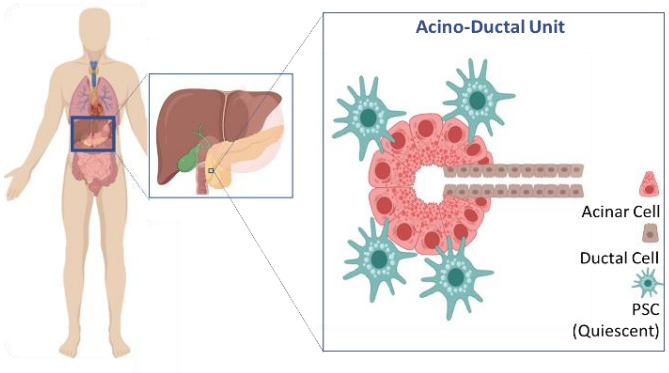


Fig. 1 *Schematic drawing representing the exocrine gland of pancreas.*

The most critical issue that makes PDAC a particularly aggressive disease, is the rapid cancer's evolution without noticeable symptoms in the early stages, resulting in a late diagnosis and poor clinical prognosis. Indeed, most of the patients present this tumor in an advanced and metastatic stage at diagnosis and only ten-twenty per cent of them are operable [2]. These negative data are also attributed to the lack of prognostic biomarkers and to the limited effectiveness of the current treatment, while the genetic complexity and the heterogeneity of the tumor hamper significant progresses in this regard.

Therefore, current research is focused on the identification of new biomarkers and the development of screening tests, in order to enable an earlier detection of tumor and to improve prognosis. Particularly, further and deeper genetic analysis of PDAC could be useful to better understand the progression of the tumor and for the identification of the primary lesions involved in this process. One of the major limitations in the comprehension of PDAC pathogenesis and in the development of novel treatments is the lack of efficacy of common preclinical models, which fail to reproduce cancer heterogeneity and to predict drug therapeutic responses in patient [5]. For many years cancer research has been based on the use of two-dimensional cell cultures that poorly recapitulate the biological complexity of the disease. Recently, more representative preclinical *in vitro* models have been investigated, such as cell spheroids and organoids, leading to the development of three-dimensional (3D) culture systems, which better mimic tumor *in vivo* conditions and allow a deeper understanding of PDAC physiopathology. However, these models represent imperfect reproductions since they do not mimic the complex glandular shape, typical of the acinar unit [6].
For this reason, the establishment of a 3D model able to mimic and recapitulate the tumoral microenvironment, in terms of both composition and morphology, is urgent needed. Here, we developed a 3D in vitro model of the acino-ductal unit which serves as a powerful tool to deeper understand the early

evolution steps of PDAC, identify novel diagnostic, prognostic and predictive biomarkers and, finally, validate innovative nanomedicine systems. To this aim, melt electrospinning technology, which combines 3D printing principles with conventional electrospinning technique [7], was employed. Then, a surface functionalization of melt-spun filaments was performed to improve hydrophilicity.

## II. MATERIALS &METHODS

The acino-ductal structure was reproduced through a melt electrospinning technology (Novaspider, Gipuzkoa, Spain) by extruding medical-grade polycaprolactone (PCL) with MW = 43 kDa in a layer-by-layer manner. The CAD model was firstly generated in SolidWorks. The fabrication process was optimized to achieve high pore interconnectivity, accuracy in geometry and precise control of pore size. Particularly, the control of printing parameters such as processing temperature, applied voltage, collector distance, collection speed, fill density and applied pressure, played a key role in the development of the complex exocrine glandular structure. An atmospheric plasma surface modification (Nadir srl) was implemented in a layer-by-layer and automatized manner in order to introduce biomimetic cues within the polycaprolactone (PCL) structure.
This 4D printing system, constituted by melt electrospinning and automatized surface functionalization, enhances the biological performances, by improving cell adhesion, proliferation and growth.

## III. RESULTS

4D-printed structures morphology was characterized by optical microscopy (Fig. 2). The images demonstrated the effectiveness of the optimization process which led to the formation of a straight filament, defect-free structures, and accurate acino-ductal geometry (Fig. 2a) . Particularly, the combination of 8 kV electric field potential and 20 $m^{-3}$ collector distance enable the jet to remain focused without

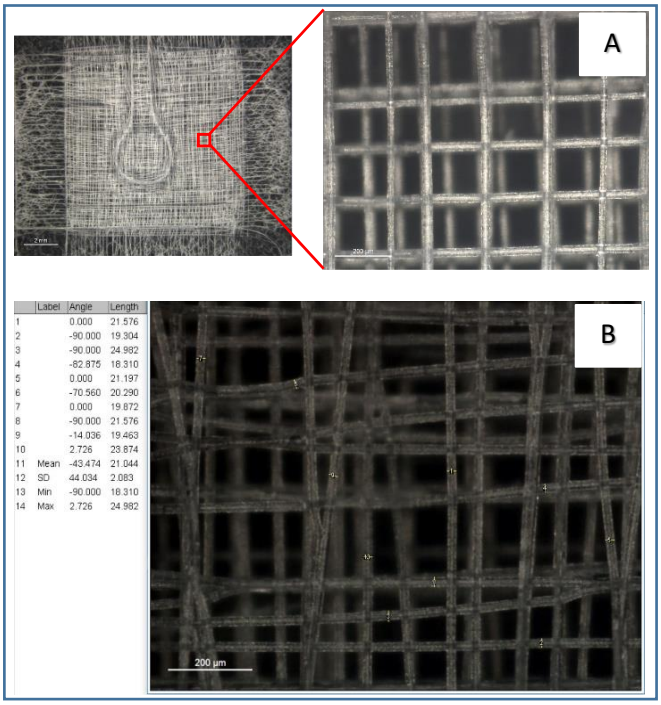


Fig. 2 *Images from optical microscopy of the structure obtained by melt electrospinning. Acino-ductal shape (A) and fibers diameters evaluation by ImageJ software (B).*

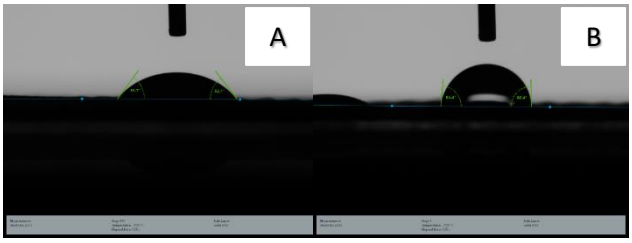


Fig. 3 *Water droplets on PCL scaffold treated by plasma (A) and on untreated PCL scaffold (B).*

arcing between the collector and spinneret, while allowing adequate distance for the fiber to cool sufficiently and thus solidified. The structures obtained result in a 10x10 mm square and the dimensions of acinus and duct diameters are 3 mm and 0.9 mm, respectively. The electrospun fibers maintain a circular section and have diameters almost uniform, with a mean value of 21 microns (Fig. 2b). The atmospheric plasma treatment led to an improvement in the wettability of PCL scaffold as confirmed by the contact angle test (Fig. 3).

## IV. DISCUSSION & CONCLUSIONS

Besides mimicking the physiological human glandular tissue, both in compositional and geometrical aspects, this model could provide a powerful tool to identify new diagnostic biomarkers and establish efficient screening tests.

The cellularized final structure will provide a model of human PDAC at early stages which could be implemented in a microfluidic system. Advanced technologies which integrate optical components in microfluidic chips could be employed for the real-time monitoring of the tumor's evolution and for performing genomic analysis [8].

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