Development of an acino-ductal unit 3D in vitro model through additive manufacturing technologies

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***Abstract*—Pancreatic ductal adenocarcinoma (PDAC) is the most common and lethal malignancy of the exocrine pancreas, primarily due to unspecific symptoms, late diagnosis, and resistance to existing treatments. The fibrotic and heterogeneous structure of the tumor microenvironment further complicates its study, underscoring the urgent need for advanced research models. In this context, the use of 3D models employing additive manufacturing techniques has emerged as a particularly promising approach. These models offer the advantage of replicating the geometry of physiological tissue, while simultaneously ensuring the customisability and reproducibility of the produced constructs. This study aims to develop a biomimetic 3D scaffold-based model of pancreatic tissue, focusing on the tumor microenvironment. To achieve this, melt electrowriting was selected as the manufacturing technique to produce microfibrous scaffolds with highly controllable fiber deposition. The scaffold design and printing process were optimized using a Python script, developed to enhance key parameters such as the fiber disposition and the efficiency of material consumption. Finally, to validate the scaffold, a cell co-culture that replicates the interactions between the tumor and the stroma has been conducted, mimicking the fundamental characteristics of the tumor microenvironment. The preliminary results exhibited an efficient proliferation and a migratory behaviour of the cells within the construct. Additionally, the scaffold design proved to be particularly advantageous in facilitating confocal imaging, simplifying the analysis of cellular behavior within the construct.**

***Keywords*—Pancreas, 3D Printing, Python, Melt-ElectroWriting.**

I. INTRODUCTION

In the last decade, a large number of pathologies connected to both endocrine and exocrine components of pancreas have been observed to arise with an increasing rate in their occurrence; in particular the pancreatic ductal adenocarcinoma (PDAC), known as pancreatic cancer, stands out as the main neoplastic disease of the exocrine pancreas and as an extremely lethal malignancy with a concerning increasing frequency in the occurrence. The danger imposed by PDAC is inherent to its low survival rate, which is connected to the late diagnosis due to lack of specific and clear symptoms before late stages and its resistance to current treatments [1], [2]. In spite of the fact that this pathology has attracted attention, the knowledge regarding its causes and specific pathological pathways is still lacking, even though it is clear that the dense fibrotic bioarchitecture of the mass and the heterogeneity of the tumor itself have a central role in the observed resistance to treatments and the consequent progression of the pathology [3].

In this scenario the conventional 2D cell culture models used to study pancreatic cancer have been found to be inconsistent to perform reliable drug screening and biotechnological studies since the tridimensional conformation of the tissue is highly relevant in its in vivo condition [4], hence 3D in vitro models acquire a crucial role in the preclinical stage and as a research tool getting closer to the physiological situation.

Among the 3D models, the scaffold-based class and the additive manufacturing (AM) techniques for their production allow to better replicate the geometry of the physiological tissue, improving the biomimicry of the final model while granting customizability of the construct [5].

Here, is described the design of a scaffold-based PDAC model through the employment of an innovative AM technique, melt electrowriting (MEW). Firstly, the scaffold design focused on mimicking the hollow structure of the adenomere surrounded by a microfibrous matrix, closely resembling the architecture of the tumor microenvironment. The entire printing process was manually designed to meet the specific requisites of the MEW technology through the development Python script, which granted precise control on fiber deposition and improved customizability of the constructs. Prior to biological validation, the optimization process ensured that the constructs met requisites for the intended application, characterizing both geometry and mechanical properties of the constructs. The final scaffolds were then employed for a co-culture experiment, combining fibroblasts, to mimic the stroma, and PDAC cells, to replicate the pathological adenomere duct.

II. MATERIALS AND METHODS

A. Scaffold Design

The design process was based on simplifying the biological structure identifying as the main component a closed-ended

tubular duct, therefore chosen as the fundamental unit of the scaffold itself.
In order to avoid the use of a sacrificial ink to allow the manufacturing of a hollow cave inside of a fibrous structure, the solution proposed in this model depends instead on the subdivision of the entire geometry into two specular shapes. Each half contains an half of the channel thus avoiding the deposition of material above the gap and maintaining the hollow feature. The two halves are then connected by a thin sheet of material that ensures proper aligning of the two halves and relative positioning, facilitating the subsequent pairing process. This design facilitates the assembly of the complete tubular structure while preserving the integrity of the hollow channel.

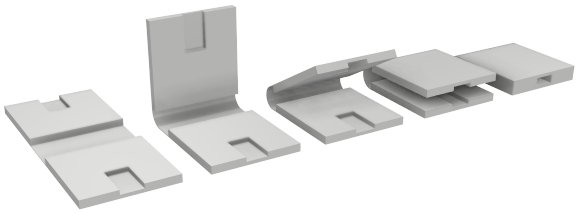


Fig. 1: Design of the foldable scaffold

By bending the connecting sheet between the two halves, as shown in Fig. 1, the assembly forms a continuous hollow channel at the center of the scaffold. This configuration replicates the tubular duct of the adenomere while maintaining the fibrous and porous architecture characteristic of the design.

B. Pathway Design - Python script

In order to instruct the MEW machine the correct pathway of the extruder, a Python script was developed to manually design the actual path for the fiber deposition, choosing an orthogonal grid pattern as the infill. The script was written using the FullControl library, which allows for precise control over the deposition process.

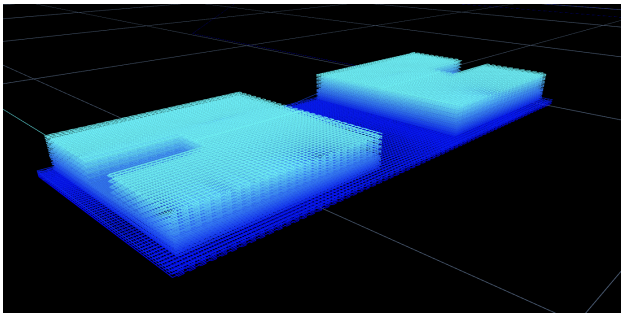


Fig. 2: Design of the extruder pathway through a Python script

C. Scaffold production and characterization

The scaffolds have been manufactured with PCL 42kDa supplied by Polysciences, provided in beads. The printing optimization was conducted using the Novaspider v5 melt electrowriting machine.
The fiber diameter and the microarchitecture of the scaffolds were analyzed using a Scanning Electron Microscope (SEM), while mechanical properties were assessed through a tensile test conducted with an MTS Q-Test TM/10 instrument, equipped with a 10N load cell.

D. Cell culture

Cellular tests have been conducted co-culturing Human Pancreatic Duct Epithelial-KRAS cells to represent the tumoral pancreatic component and Human Foreskin Fibroblasts (HFF1) to mimick the stroma.
HFF-1 cells were expanded in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 15% Fetal Bovine Serum (FBS), 1% Penicillin/Streptomycin (P/S), and 2% L-Glutamine in a humidified incubator at 37°C with 5% CO_2. Cells were collected and seeded onto open scaffolds at a density of 3×10^4 cells in 40 µl per half-scaffold, distributed into four 10 µl drops for uniform seeding. The scaffolds were incubated open for 2 hours to allow even distribution, then closed and cultured for 14 days.
During this phase, transwells without membranes were used to stabilize the scaffolds, preventing disassembly.

HPDE-KRAS cells were subsequently expanded in RPMI medium supplemented with 10% FBS, 1% P/S, and 1% L-Glutamine, then seeded at a density of 1.5×10^4 cells in 10 µl per half-channel. After the HFF-1 seeding, scaffolds were reopened for HPDE-KRAS cell seeding, incubated open for 1 hour, and then closed. Again, transwells without membranes were used to stabilize the scaffolds during this step, ensuring that the scaffolds remained intact throughout the cell seeding and culture. Co-culture of HFF-1 and HPDE-KRAS cells was then maintained in DMEM/F12 medium supplemented with 15% FBS.

SEM was employed to evaluate the morphology of the cells and their interaction with the scaffold. This imaging technique provided detailed insights into the cellular adherence and organization on the fibrous structure of the scaffold.

Cell staining involved immunofluorescence using HECD-1 (ThermoFischer, USA) antibody to distinguish HPDE-KRAS cells from HFF-1 in co-culture, along with DAPI (D1306, Invitrogen, USA) and Phalloidin (P3457, Invitrogen, USA) staining to visualize nuclei and cytoskeleton.

III. Results and Discussion

The results of the printing parameters' optimization are shown in Fig. 3.
The MEW technology proved to be a highly effective tool for scaffold fabrication, achieving enhanced resolution and precision compared to conventional fabrication methods.

Notably, the fiber diameters produced were significantly smaller than those achieved using techniques like fusion deposition modeling, with a mean fiber diameter of 21.382 $\pm$ 3.527 μm, as measured from 50 fibers across three different samples using ImageJ.
The tensile test, conducted on three samples as well, identified a mean stiffness of 18.00 $\pm$ 4.05 MPa.

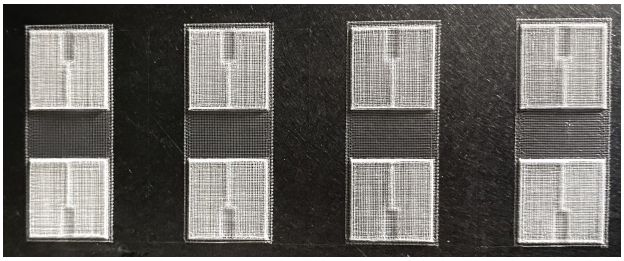


Fig. 3: Optimized scaffolds produced via melt electrowriting

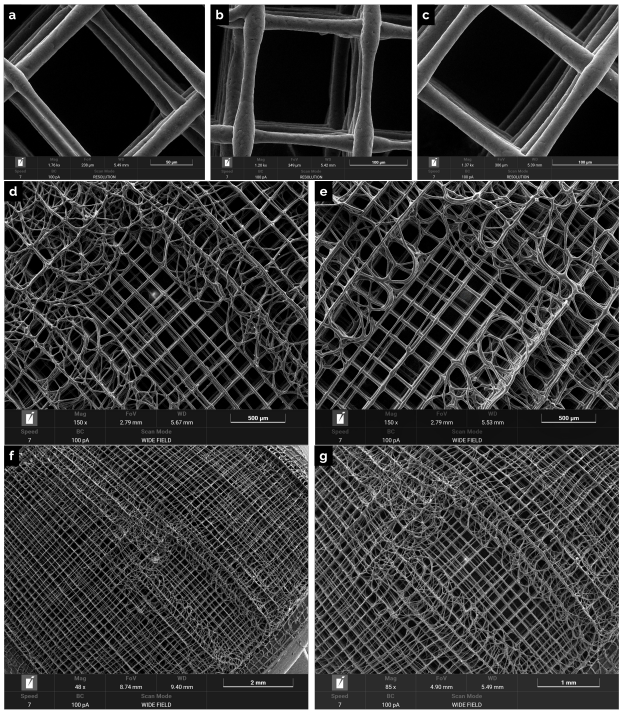


Fig. 4: SEM images of the optimized scaffold

SEM analysis of scaffolds after 14 days of cell culture revealed extensive proliferation and colonization, particularly by fibroblasts interacting with the pure PCL fibers. As shown in Fig. 5 (e,f), the scaffold's surface was densely populated with cells, demonstrating successful adherence and integration with the fibrous structure. Higher magnification images (Fig. 5 a-d) provided detailed views of cell-fiber interactions, highlighting cell spreading both on the horizontal plane and in the vertical direction, which indicate a strong affinity between the scaffold material and the cells.
To further evaluate the cell culture dynamics, scaffolds were harvested at five time-points (48h, 72h, 7d, 10d, and 14d), with

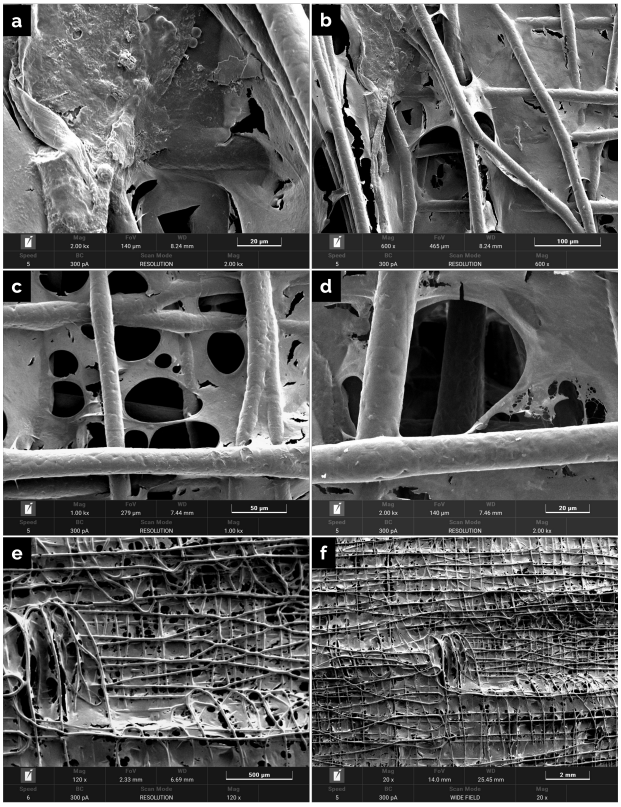


Fig. 5: SEM images of the colonized scaffold

Day 0 corresponding to HPDE-KRAS cell seeding. Immunohistochemical analysis performed using confocal microscopy allowed visualization of cell behaviour within the scaffold over time. To facilitate observation, scaffolds were opened to expose the interior channel, where E-cadherin (specific to HPDE-KRAS cells) was stained magenta, nuclei were stained blue with DAPI, and the cytoskeleton was labeled green with Phalloidin. When performing the analysis, the scaffolds were opened to directly observe the channel's interior, revealing E-cadherins (HPDE-KRAS specific) marked in magenta, nuclei in blue, and the cytoskeleton in green.
To analyze the spatial distribution of cells along the scaffold's vertical axis, two focal planes were selected: the "channel plane" (the seeding plane for HPDE-KRAS cells, Fig. 6a) and the "superior plane" (the seeding plane for HFF-1 fibroblasts, Fig. 6b). This approach allowed a detailed evaluation of cell behavior both inside the channel (the 'channel plane') and on the exterior surface of the scaffold (referred to as the 'superior plane'). Notably, this 'superior plane' corresponds to the middle layer of the scaffold's bulk structure during cell culture, where HFF-1 fibroblasts were seeded and distributed across the fibrous network.
This distribution underscores the role of the scaffold architecture in promoting cell compartmentalization and interaction, closely mimicking the tumor-stroma interface in pancreatic ductal adenocarcinoma.

The overall response exhibited by the HFF-1 and HPDE-

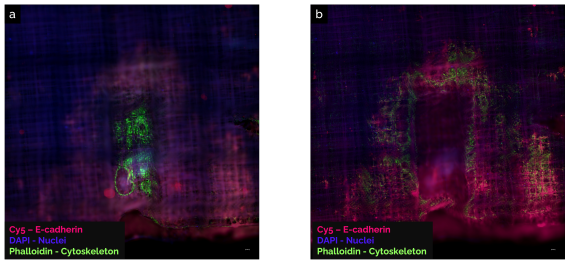


Fig. 6: Representation of the two focal planes chosen for the evaluation of the cell co-culture, a) focused on the channel plane, b) on the superior plane

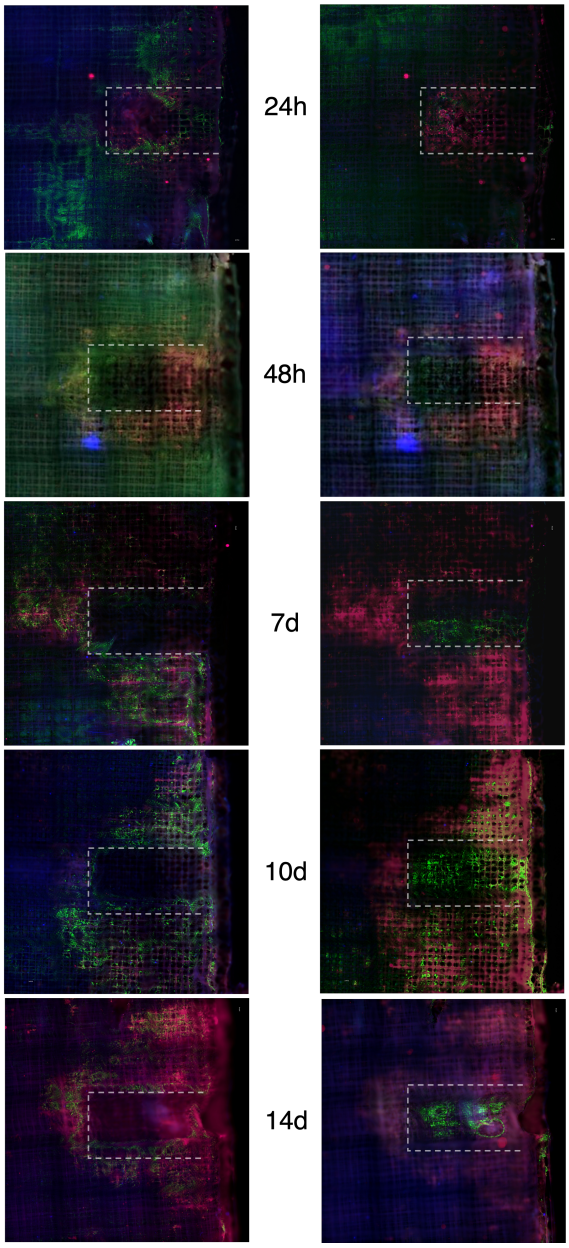


Fig. 7: Progression of the spatial distribution of the two cell types in the scaffold area, obtained merging magenta, blue and green channels. The position of the scaffold's channel is highlighted by a dashed line in order to better recognize its location.

KRAS cells was strongly positive, demonstrating sustained viability throughout the entire culture period (14 days of monoculture and 14 days of coculture). HFF-1 cells formed a dense network within the fibrous matrix, creating a supportive microenvironment, while HPDE-KRAS cells exhibited notable proliferation and migratory behavior, actively invading the HFF-1 colonized regions and fostering interaction between the two cell types under co-culture conditions.
However, during the culture period, a fully developed channel structure was not observed.

The scaffold's design and its ability to be opened for direct observation of the channel component were critical for analyzing pancreatic cell behavior. During analysis, this configuration allowed easy access to the inner channel and the surrounding fibrous matrix, eliminating the need for embedding or slicing the construct, thus significantly simplifying the observation process.

IV. Conclusion

In conclusion, the results obtained underscore the potential of the described approach for future applications in pancreatic tissue modeling. The scaffold design demonstrated both versatility and practicality, facilitating the manufacturing process without requiring sacrificial materials and enabling straightforward access to the internal channel for imaging purposes.

The cellular experiments revealed robust proliferation and colonization of HFF-1 cells within the 3D PCL structures, as well as a successful interaction with HPDE-KRAS cells during co-culture. This highlights the scaffold's capacity to support a biomimetic tumor-stroma microenvironment.

Although a fully defined channel structure was not observed, the behavior of the cells suggests that this approach holds promise for replicating the adenomere's architecture. Further refinements to the scaffold design and culture protocols could enhance its fidelity as a model system, expanding its potential applications in cancer research and drug screening.

References

[1] I. X. Hu, Y. Y. Lin, C. F. Zhao, W. B. Chen, Q. C. Liu, et al., Pancreatic cancer: A review of epidemiology, trend, and risk factors. World journal of gastroenterology, World journal of gastroenterology, 27:4298–4321, 7 2021

[2] W. Park, A. Chawla, and E. M. O'Reilly. Pancreatic cancer: A review. JAMA, 326:851–862, 9 2021

[3] K. Szymoński, K. Milian-Ciesielska, E. Lipiec, and D. Adamek. Current pathology model of pancreatic cancer. Cancers 2022, Vol. 14, Page 2321, 14:2321, 5 2022.

[4] T. Saydé, O. El Hamoui, B. Alies, K. Gaudin and G. Lespes, et al. Biomaterials for three-dimensional cell culture: From applications in oncology to nanotechnology. Nanomaterials 2021, Vol. 11, Page 481, 11:481, 2 2021.

[5] A. Eltom, G. Zhong, and A. Muhammad. Scaffold techniques and designs in tissue engineering functions and purposes: A review. Advances in Materials Science and Engineering, 2019, 2019.