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Physico-Chemical Characterization of the Tumour Microenvironment of Pancreatic Ductal Adenocarcinoma

Alejandro Calderón-Villalba[1]; Pilar Alamán-Díez[1], Elena García-Gareta[1,2,3] ,Jose Manuel García-Aznar[1,2]

1 Multiscale in Mechanical & Biological Engineering Research Group, Aragon Institute of Engineering Research (I3A), Universidad de Zaragoza, Mariano Esquillor s/n, 50018, Zaragoza, Spain.
2 Aragon Institute for Health Research (IIS Aragon), Miguel Servet University Hospital, Zaragoza, Aragon, Spain
3 Division of Biomaterials & Tissue Engineering, UCL Eastman Dental Institute, University College London, London, United Kingdom

Introduction

- **Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent form among all pancreatic cancer types.**
- **Limited response to conventional clinical treatments, which include chemotherapy, surgery and radiotherapy.**
- **PDAC displays a non-immunogenic, immune-suppressive and therapy-resistant microenvironment, largely attributed to its intrincate tumor microenvironment (TME).**

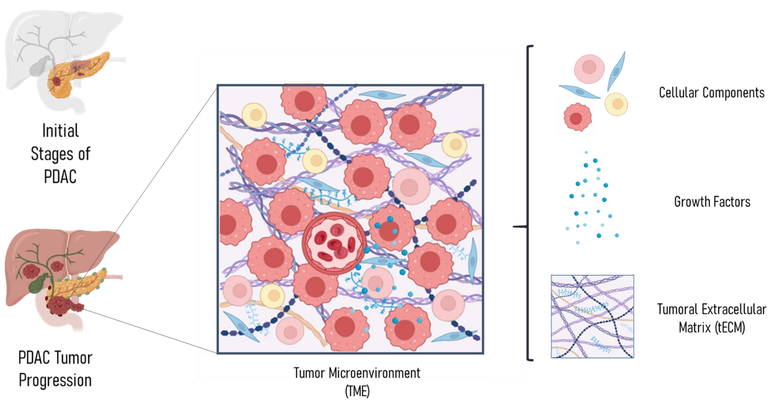


Figure 1. PDAC progression and tumoral microenvironment (TME) main components.

Aim and Experimental Design

- **To characterize the physico-chemical properties of the PDAC tumor microenvironment using patient-derived xenografts (PDX) and advanced decellularization techniques. By understanding these properties, novel therapeutic strategies may be developed to target PDAC more effectively and improve patient outcome.**

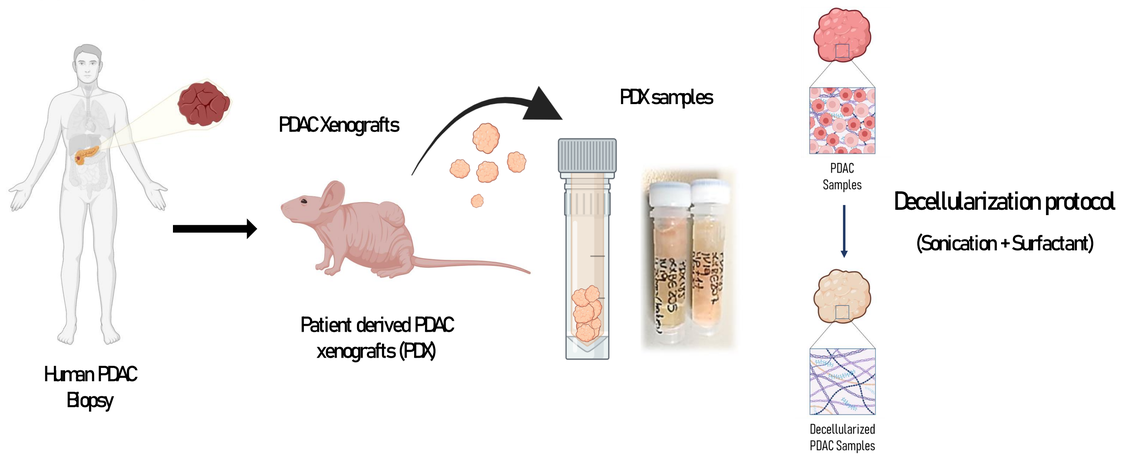


Figure 2. General scheme of PDX murine models generation and sample processing

Results

1. Quantification of decellularization.

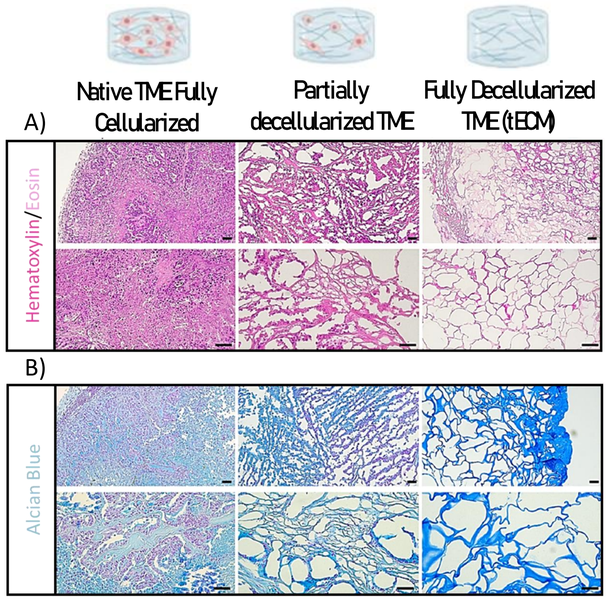


FIG.1 (A) Hematoxylin-eosin staining (matrix in pink and cells as purple dots) and (B) Alcian blue stainning (GAGs in blue and cells in pink/purple dots) of native, partially decellularized and fully decellularized PDX samples. Histological stainings showed abundant desmoplasia with extensive protein deposition recapitulating the pattern found in human PDAC TME. Alcian blue staining revealed the presence of GAGs in the ECM even after de decellularization process. Scale bar – 100 µm

2. dsDNA quantification.

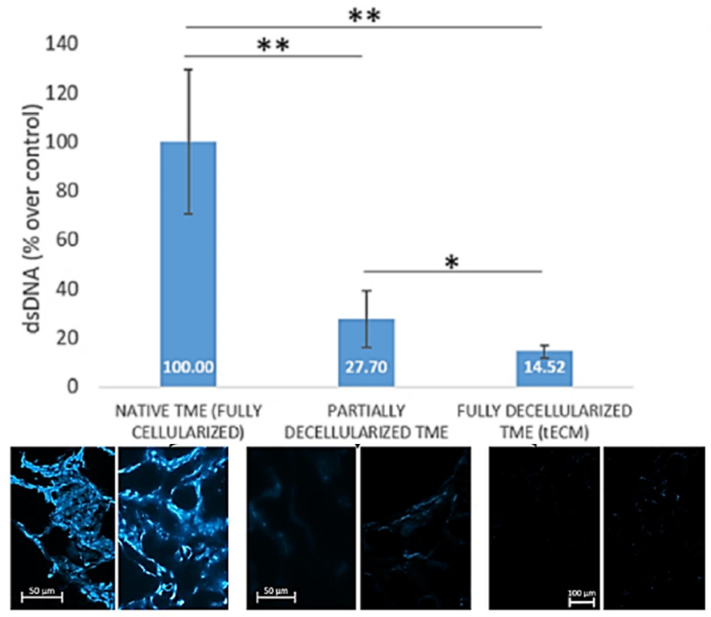
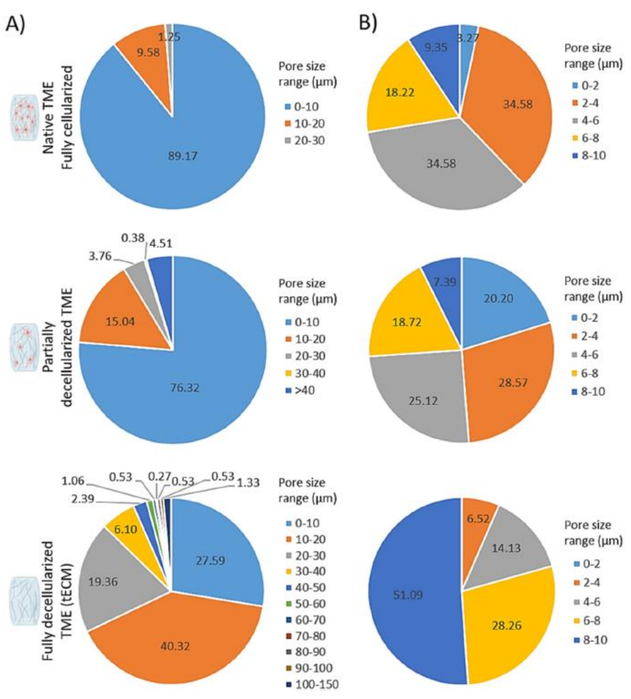


FIG.2 Quantification of double stranded DNA (dsDNA) was performed on DAPI stainings of histological sections showing a significant reduction in dsDNA content between the native TME, partially and fully decellularized tissue. Residual blue fluorescence can be observed in the fully decellularized samples which may be indicative of the presence of cellular debris as well as residual dsDNA trapped in the tECM after decellularization.

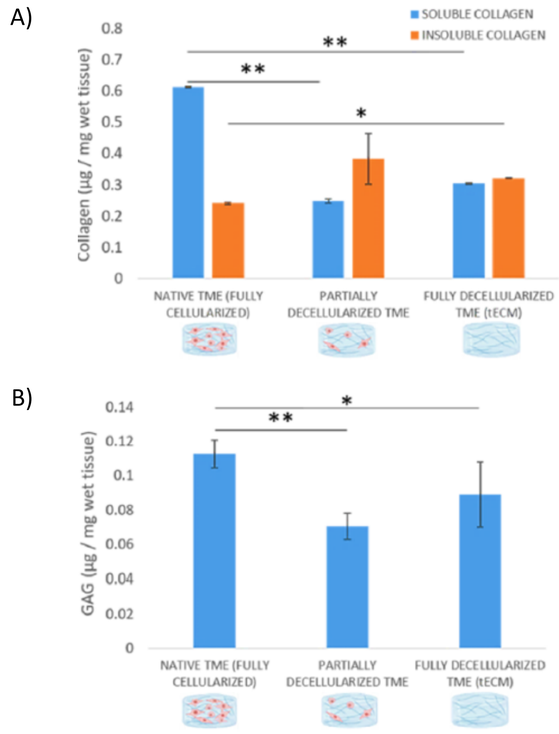
3. Pore size distribution

FIG.3 Superficial pore size analysis based un SEM images of PDAC samples. (A) All pores. B) Breakdown of pores in the 0 to 10 µm range of size. Our results showed that the decellularization released residual or accumulated stresses exerted by the cells to the TME, thereby opening the porous tECM network and increasing permeability to liquids, drugs, large molecules and cells used as therapeutic or delivery agents.



4. Collagen and GAGs quantification.

FIG.4 Collagen (A) and GAGs (B) content of native, partially and fully decellularized samples were measured using comercial kits. Quantification of total collagen showed a significant reduction in the amount of soluble collagen during decellularization process something expected since it is not heavily crosslinked to ECM. Insoluble collagen seems to increase in the decellularized samples however this is due to the normalization by wet weigh. Regarding GAG quantification, we observed a significant reduction with decellularization which was expected since about 30% of GAGs are asociated with the cell membrane and thus, is eliminated with the celular fraction.



Conclusions

The incremented permeability and pore size observed during decellularization reveal that the cellular components of the TME exert a relevant contractile role in this particular tumor, probably due to a high presence of cancer associated fibroblasts (CAFs) which also contribute to the desmoplastic deposition of tECM proteins. This research offers a potential therapeutic strategy based on the application of therapeutic agents with small size such as small drugs or therapeutic cells and, although further work is required this opens a new innovative therapeutic field to develop new strategies in cancer treatment.

QR to the Article

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