Bioengineered 3D Tumor Microenvironment Models for Screening Anti-Cancer Therapies

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Introduction

Human tumors exhibit high resistance to current therapies, largely driven by the complexity of the tumor microenvironment (TME), which regulates disease progression and treatment response. In pancreatic ductal adenocarcinoma (PDAC), a dense desmoplastic stroma and immunosuppressive milieu impair immune cell infiltration and therapeutic efficacy. Reproducing the biological and biophysical complexity of tumors in vitro remains challenging, particularly when aiming for miniaturized, reproducible, and scalable platforms suitable for high-throughput screening. Advanced 3D tumor models integrating stromal and immune components and recapitulating the native TME are therefore essential to better predict therapeutic responses.

Materials and Methods

Miniaturized 3D spherical tumor platforms were developed using superhydrophobic surfaces, enabling the reproducible generation of uniform tumor microtissues. The system was based on a compartmentalized cancer-on-a-bead architecture, where PANC-1 cells were encapsulated in a GelMA hydrogel (5% w/v) core and cancer-associated fibroblasts (CAFs) were embedded in a surrounding GelMA (5% w/v)/HAMA (1% w/v) ECM-mimetic stromal shell. Monotypic spheroids and stratified tumor-stroma spheroids (STAMS) were used as controls to assess the contribution of stromal and ECM components for microtumor aggressiveness and resistance. 3D tumor models were characterized through qPCR analysis, and co-cultured with dendritic cells, macrophages, and T lymphocytes to evaluate tumor-stroma-immune interactions. Immune phenotyping was performed by flow cytometry, cytokine secretion by ELISA, and the platform was further validated for immunotherapy screening using anti-PD-1 treatment.

Results and Discussion

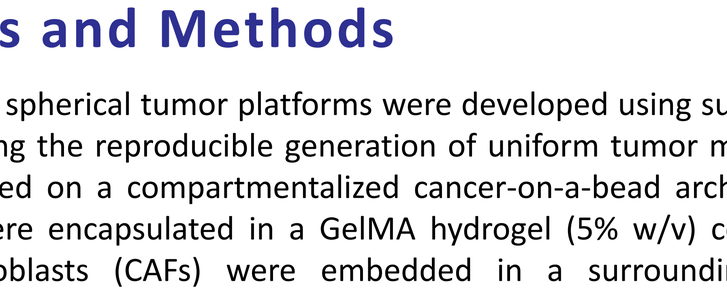


Figure 1. Gene transcriptional signature of tumor-stroma 3D in vitro models. Total mRNA was extracted, reverse transcribed, and the transcriptional levels of key immunoregulatory and stemness-related genes were analyzed by quantitative PCR (qPCR). Log2 mRNA fold changes for each gene were calculated relative to the maximum transcription levels observed across analyzed samples after normalization with reference gene HPRT1. The heatmap displays the mean values from three independent experiments.

Gene expression analysis showed that the incorporation of CAFs in 3D tumor-stroma models led to a marked **upregulation of genes associated with desmoplasia, ECM remodeling, and immunomodulation**, reflecting the establishment of a fibrotic and immunosuppressive tumor microenvironment. Tumor-stroma models, particularly cancer-on-a-bead platforms, also showed **increased expression of stemness and drug resistance markers** (CD24, CD44, CD133, ABC transporters), as well as pro-tumorigenic and immunosuppressive mediators (VEGF, PD-L1, IDO, PTGS2).

Overall, the presence of CAFs and ECM components promotes the establishment of a highly immunosuppressive and physiologically relevant TME, capable of impairing dendritic cell (DCs) function, driving macrophage polarization toward M2 phenotypes, and inducing T cell dysfunction and exhaustion.

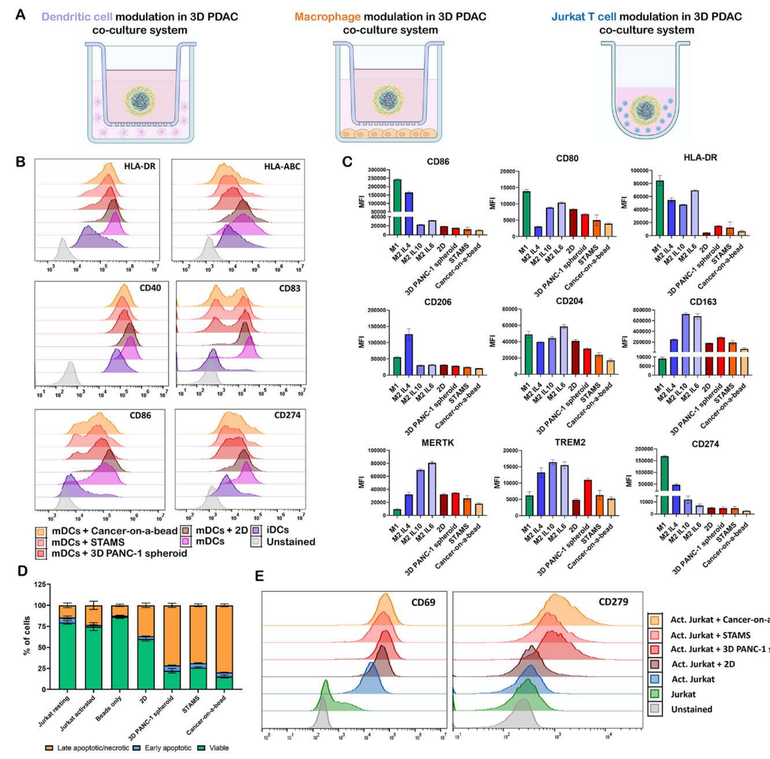


Figure 2. Evaluation of immunomodulatory effects of tumor-stroma 3D models on immune cells. (A) Schematics representation of co-culture 3D PDAC models with immune cells. (B) Phenotypic analysis of DCs after co-culture with 3D models. DC expression of maturation markers HLA-DR, HLA-ABC, costimulatory CD40, CD83, CD86, and co inhibitory CD274 were analyzed by flow cytometry. (C) Analysis of macrophages polarization phenotype. The expression of key M1 and M2 markers were analyzed by flow cytometry. (D–E) Impact of 3D tumors on lymphocyte exhaustion and viability. Activated Jurkat cells were co-cultured with different PDAC platforms and apoptotic/necrotic state, as well as the expression of exhaustion markers were addressed by flow cytometry.

Co-culture of 3D PDAC tumor-stroma models with immune cells revealed a strong **immunosuppressive effect** across multiple immune populations. Dendritic cells exposed to these models showed significant downregulation of maturation and costimulatory markers (CD80, CD86, CD40, CD83, HLA-DR), indicating impaired antigen presentation and reduced capacity to activate T cells. Macrophages co-cultured with tumor models exhibited reduced M1 markers (CD80, CD86, CD274) and increased M2 markers (CD163, CD204), consistent with polarization toward a pro-tumoral phenotype, particularly in stratified and cancer-on-a-bead models. In parallel, T lymphocytes exhibited reduced viability, increased apoptosis, and upregulation of exhaustion markers (PD-1, CD69), with stronger effects in cancer-on-a-bead systems.

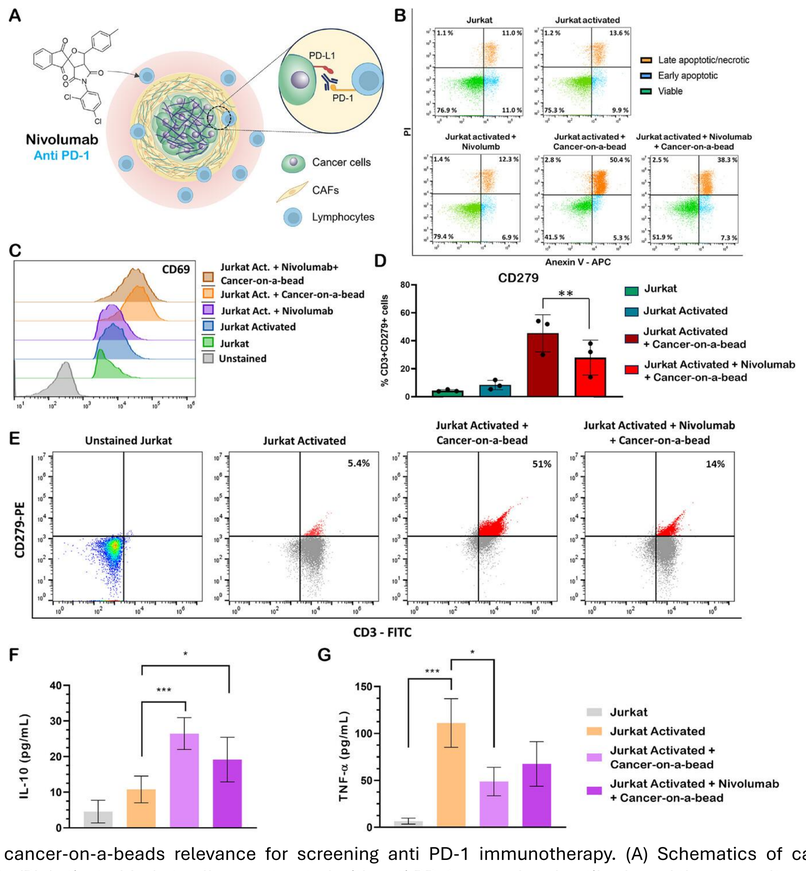


Figure 3. Evaluation of cancer-on-a-beads relevance for screening anti PD-1 immunotherapy. (A) Schematics of cancer-on-a-bead models treatment with Nivolumab. (B) Activated Jurkat cells were treated with anti PD-1 monoclonal antibody and then co-cultured with cancer-on-a-bead model for an additional 24 h. T cells apoptosis/necrosis was analyzed with Annexin/PI staining by flow cytometry. (C–E) Expression of T cells activation marker CD69 (C) and exhaustion marker CD279 (D and E) markers were analyzed by flow cytometry. (F and G) ELISA-based quantification of IL-10 and TNF-α in cell culture supernatant of the different conditions.

Nivolumab-mediated PD-1 blockade improved T cell viability and reduced apoptosis in cancer-on-a-bead models, partially restoring T cell function within the immunosuppressive TME. This was associated with increased CD69 and decreased PD-1 expression, alongside reduced IL-10 and partial recovery of TNF-α levels.

Conclusions

Bioengineered stratified 3D tumor-stroma models recapitulate key structural and immunological features of PDAC, enabling the study of tumor-immune interactions and immunotherapy responses. These platforms bridge the gap between in vitro and in vivo systems, supporting translational research.

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