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A 3D Bioprinted Colorectal Cancer Model Using Tumor-Derived dECM, Apelin-13 and Collagen I to recapitulate Tumor-Stroma Interactions

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Introduction

Colorectal cancer remains one of the leading causes of cancer-related mortality worldwide, with complex tumor–stromal interactions and high interpatient variability posing significant challenges for effective treatment development. Traditional two-dimensional (2D) culture models and animal studies often fail to replicate the intricate architecture, biochemical cues, and cellular heterogeneity of the human tumor microenvironment (TME), limiting their translational relevance. In recent years, three-dimensional (3D) tumor models have emerged as promising platforms for advancing personalized cancer research, enabling more physiologically relevant drug screening and immunological investigations.

One particularly promising approach involves the use of decellularized extracellular matrix (dECM) derived from tumor tissues, which preserves native biochemical and structural cues. However, a major bottleneck remains: the limited efficiency of recellularization within such scaffolds, which hampers their biological fidelity and functional relevance.

To overcome this limitation, our study proposes a novel biofabrication strategy that integrates dECM powder from colon tumors, apelin--13 (a peptide known to modulate angiogenesis, proliferation and tissue remodeling), type I collagen (COL-I), and cancer-associated fibroblasts (CAFs) into a customized 3D bioprinting system. This method aims to generate a bioactive, biomimetic scaffold that not only enhances cellular attachment and proliferation but also closely mimics the colon cancer TME.

By evaluating the recellularization efficiency and biological functionality of this engineered scaffold, we aim to establish an advanced in vitro 3D model of colorectal cancer. Such a model holds great promise for personalized medicine, particularly in high-throughput drug screening and immune profiling, potentially accelerating the development of more targeted and effective therapeutic strategies.

Methods & study design

Preparation of Decellularized ECM (dECM):
Colon cancer tissues were decellularized using a combined chemical and enzymatic protocol optimized to preserve the structural integrity of the extracellular matrix while removing all cellular components. The efficiency of decellularization was validated by DAPI staining (for nuclear content) and hematoxylin and eosin (H&E) staining (for histoarchitecture). The resulting acellular matrix was lyophilized and milled into a fine ECM powder for use in scaffold fabrication.

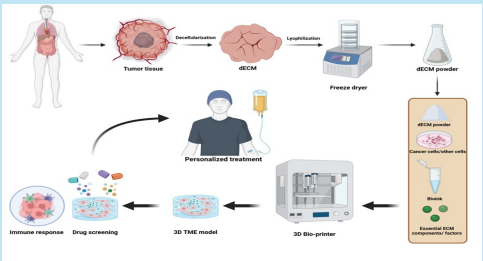
Immunofluorescence (IF) Staining of Native Tumor Tissue:
To evaluate the expression and localization of key matrix and signaling components, immunofluorescence staining was performed on native colon cancer tissue sections. Samples were stained using primary antibodies against collagen type I and apelin-13, followed by incubation with fluorescently labeled secondary antibodies. Nuclei were counterstained with DAPI, and slides were imaged using a fluorescence microscope to assess the distribution of these markers within the tumor microenvironment.

Cytotoxicity and Proliferation Assays:
Biocompatibility of the dECM powder was assessed by treating LoVo colorectal cancer cells with varying concentrations, followed by MTT assay to evaluate cell viability. The influence of apelin-13 and COL-I on LoVo cell proliferation was further examined using standard proliferation assays over a defined culture period.

Cell Migration Assay:
A modified agarose spot assay was used to assess the chemotactic response of LoVo cells to apelin-13 and COL-I, quantifying directional cell migration under controlled in vitro conditions.

3D Bioprinting of Tumor Scaffolds:
Bioactive 3D scaffolds were fabricated using an extrusion-based 3D bioprinter, incorporating a bio-ink composed of dECM powder, LoVo cells, apelin-13, COL-I, and laminin, with or without CAFs. Scaffolds were printed into defined geometries and cultured under physiological conditions to promote cellular integration and matrix remodeling.

Evaluation of Scaffold Performance:
Post-printing evaluation of cell viability, distribution, and scaffold integration was conducted using a combination of live/dead staining, MTT assays, and H&E staining at multiple time points to monitor cell behavior within the constructs.



Conclusion

The 3D bio-printed scaffold, comprising dECM, apelin-13, collagen type I, and laminin bio-ink, LOVO and CAF cells provided a supportive microenvironment that enhanced cell viability and localization, demonstrated by live/dead staining and MTT results. These findings suggest that this bioactive scaffold design could serve as an effective 3D model for studying tumor cell interactions and tissue regeneration in vitro. By supporting cell proliferation, migration, and viability, this approach holds potential for advancing tissue engineering applications and developing more effective cancer research models that more closely mimic the tumor microenvironment.

Results

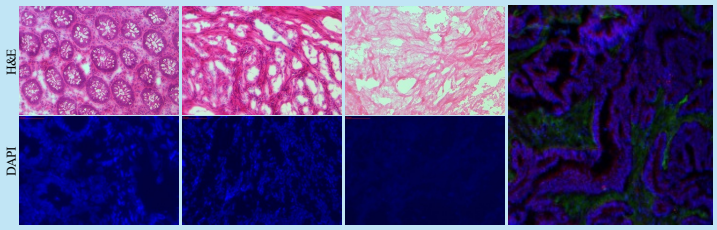
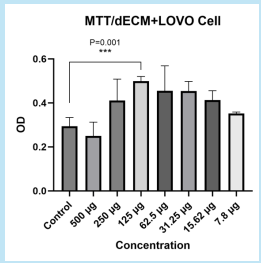


Fig 1. Our decellularization protocol effectively removed cellular material, as confirmed by DAPI and H&E staining. IF staining on the tumor tissue indicated the localization of apelin-13 (alex594-red), collagen (alex488-green), and cells (DAPI).

Fig 2. MTT assay results demonstrated that 125 µg of decellularized ECM (dECM) significantly enhanced LoVo cell viability compared to the control group ($p = 0.001$), indicating a bioactive effect at this concentration. Cell viability peaked at 125–62.5 µg, while higher concentrations (250–500 µg) showed reduced viability, possibly due to matrix density–related stress. Lower doses (≤15.62 µg) also showed a declining trend. These findings suggest an optimal concentration range (62.5–125 µg) for promoting colorectal cancer cell growth, supporting its use in 3D bio-ink formulation.



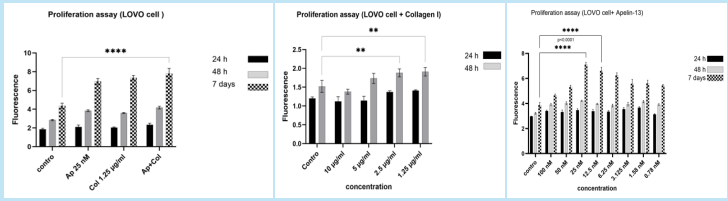


Fig 3. The combination of Apelin-13 and Collagen (Ap+Col) results in a significant increase in proliferation or total DNA content, suggesting a synergetic effect on the cells.

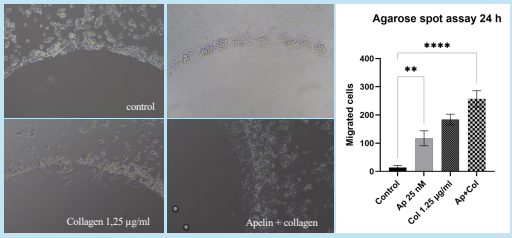
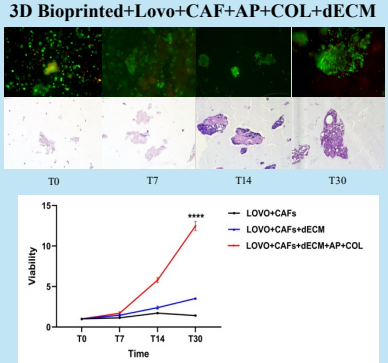


Fig 4. The agarose spot assay results demonstrated that both Apelin and collagen, at defined concentrations, are capable of promoting cell migration. Notably, the combination of collagen and Apelin led to a marked enhancement in migratory activity ($P<0.05$).

Fig 5. As depicted in Figure1, the co-culture of LOVO cells and CAFs(LOVO + CAFs group, black line) exhibited a modest increase in viability over the 30 day period. The inclusion of dECM in the scaffold (LOVO + CAFs + dECM group, blue line) appeared to slightly enhance cell viability compared to the LOVO + CAFs group, particularly at the later time points (day 14 and day 30). Notably, the incorporation of dECM, apelin-13, and collagen type I (LOVO + CAFs + dECM + AP + COL group, red line) resulted in a significantly elevated level of LOVO cell viability compared to the other two groups. Starting from day 7, this group demonstrated a markedly steeper increase in viability, reaching a peak at day 30. Statistical analysis (indicated by****) revealed a highly significant difference ($p < 0.0001$) in viability at day 30 between the LOVO + CAFs + dECM + AP + COL group and both the LOVO + CAFs and LOVO + CAFs + dECM groups



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