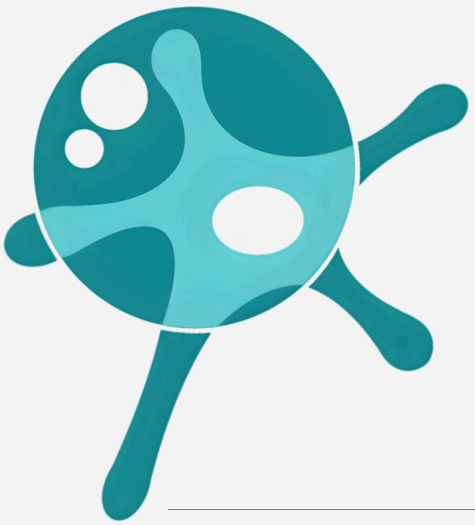
Technical white paper, 2026



TissueTinker organ-specific bioprinting system

Supercharging preclinical cancer research with 3D printed human tumors

Executive Summary 1

More than 8 in 9 oncology drug candidates fail on the way to seeking FDA approval. Why does this happen? Evidence shows that these clinical trial failures can be largely attributed to the lack of physiological relevance in preclinical screening models. Animal models, traditional 2D cultures, and synthetic 3D scaffolds are simply not cutting it, as they fail to replicate the complex biochemical and mechanical signaling of the human tumor microenvironment. Drug developers and regulators must now turn to new approach methodologies (NAMs) as a way to bridge the translational gap between early-stage drug screening and human results, and to meet new FDA and EMA guidance regarding animal models.

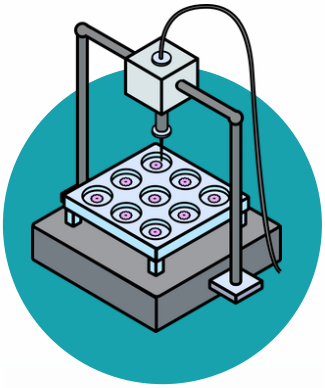
TissueTinker Bio (TTB) is solving for this challenge by building a tumor modeling system using 3D bioprinting, thereby providing pharmaceutical and biotech companies with an all-in-one drug development tool that, with just the push of a button, delivering rapid, translatable, low-cost results via our 3D-printed living human microtissue technology. The backbone of our solution is in our family of proprietary tissue-specific bioactive bioinks, which are designed to restore the "biochemical conversation" between malignant cells and their surrounding stroma. By utilizing organ-specific, tissue-derived matrices, our platform allows for the fabrication of 3D models that exhibit superior phenotypic fidelity and predictive drug responses compared to generic alternatives.

TissueTinker Workflow 2

Advanced tissue models shouldn't need complex workflows or specialty culture conditions. Yet, the current solutions on the market require a level of tissue engineering knowledge that poses a barrier for many biotech companies when it comes to integrating them into their pipeline. TissueTinker's system is purpose-built to integrate seamlessly into existing laboratory workflows, meaning that all of your assays, microscopy techniques, and culture media stay the same.

2.1. End-to-end 3D Cell Culture Solution

TTB Bioprinter



Low-cost bioprinter, designed for easy of use. No setup required; from box to first print in minutes

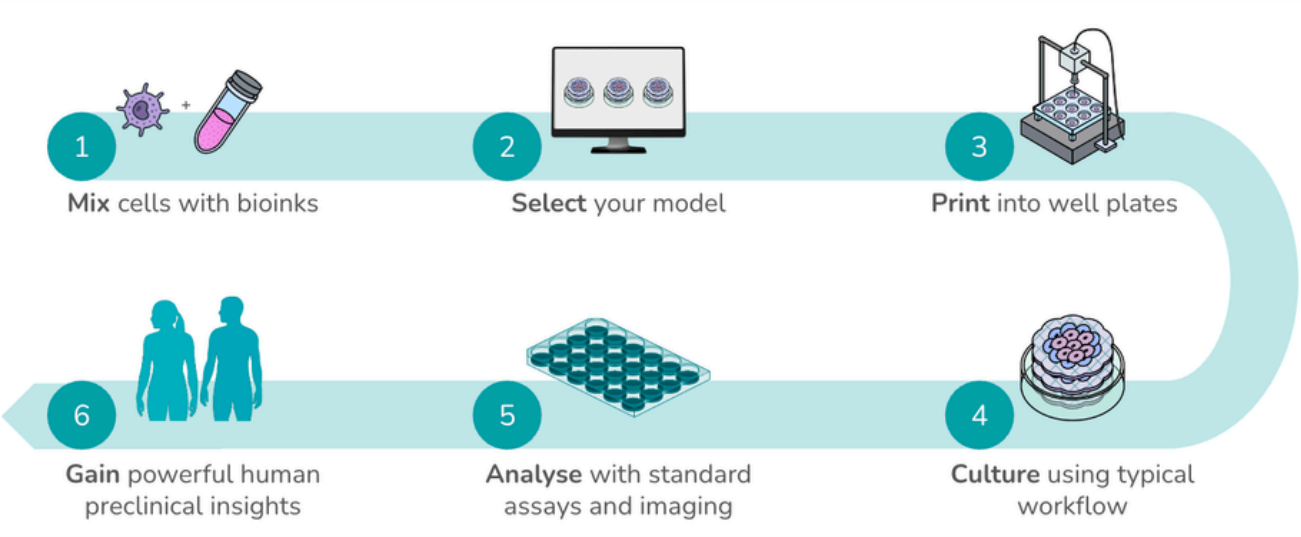
TTB Digital Model Library

Software library of ready-to-print tissue geometries, pre-designed for different experimental needs

TTB-Series Bioinks

Scaffolding materials that replicate the mechanical and biochemical properties of the extracellular matrix

2.2. Built for Seamless Lab Integration



2.3. Compatible Technologies and Products

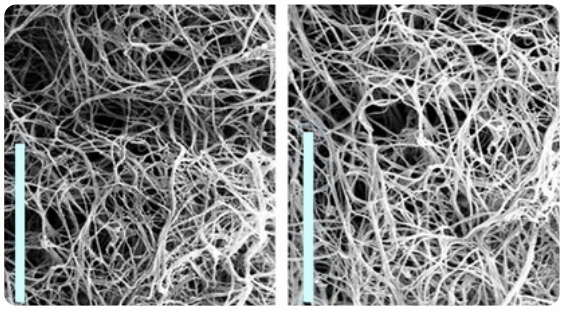
TissueTinker is more than just a standalone tumor modeling solution; it's an additive biomanufacturing system that connects and enhances the entire preclinical ecosystem. TissueTinker is not only compatible with standard culture plates and cell lines, but also with other NAM technologies, including microfluidic organ-on-a-chip technology, organoids, and patient explants.

	TissueTinker		TissueTinker + Other NAMs		
Compatible Products	Glass and plastic well plates (6, 12, 96, etc.)	Commercial cell lines (cancer cells, fibroblasts, etc.), patient derived cells, stem cells	Organoids and Spheroids	Microfluidic systems	Patient explants
Use Case	Advanced tumor models with scalable complexity based on study needs		Encapsulate constructs to emulate stromal layers	Print linked microtissue systems or apply flow conditions	Encapsulate explants with stroma or print surrounding healthy tissue

The TTB-Series Bioink Family 3

3.1. Biochemical Authenticity

Unlike reconstituted collagen, TTB-Series bioinks retain a complex proteomic signature. TTB-Series bioinks are validated by mass spectrometry analysis to ensure the presence of factors that support and guide physiologically relevant cell growth and organization.



- **Structural Proteins:** Collagens I, III, and IV in physiological ratios.
- **Adhesion Molecules:** Laminin and Fibronectin, essential for integrin-mediated signaling.
- **Soluble Factors:** Glycosaminoglycans (GAGs) that modulate cytokine gradients and cell-to-cell signaling.

Fig. 1: Topological Characterization Type-T bioink. Scanning electron microscopy images of Pt coated columns.

3.2. Tunable Mechanical Properties & 3D Printability

A critical barrier in complex model fabrication is the "printability-viability" trade-off. The TTB-Series bioinks exhibit unique mechanical properties:

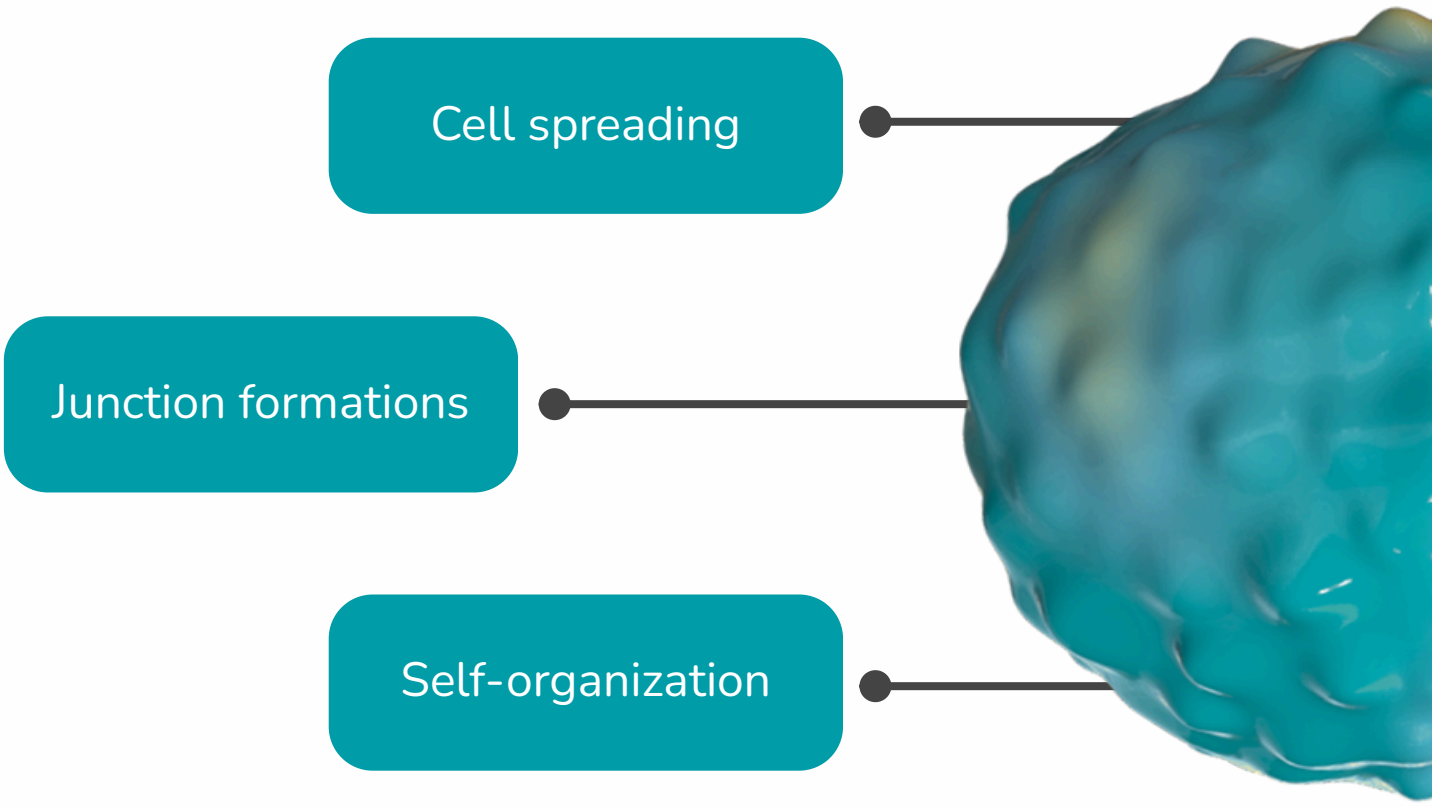
Shear-Thinning Behavior: Our bioinks flow easily under pressure during extrusion, protecting encapsulated cells from high shear stress.

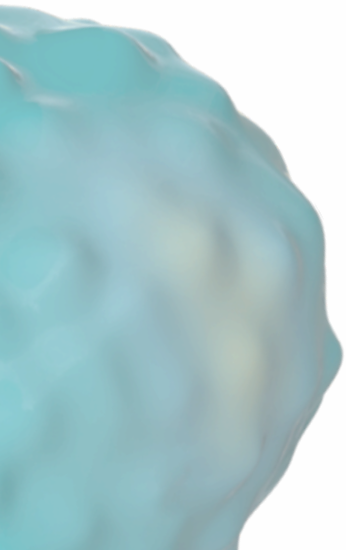
Thermo-Chemical Gelation: Upon deposition at physiological temperatures (37°C), the matrix undergoes rapid fibrillogenesis aided by a crosslinker, creating a stable, self-supporting 3D structure without the need for UV exposure.

3D bioprinting enables the consistent and reproducible fabrication of models in a high-throughput manner. This supports multiple parallel testing conditions while mitigating errors in dimensions and cell density associated with manual fabrication techniques. Unlike other liquid handlers, 3D bioprinting supports material extrusion in a broad range of viscosities (30 mPa s−1 to > 6 × 10^7 mPa s−1), and at high cell densities (<10^6 cells/mL) closer to those of human tissues.

3.3. Tissue-Specific Mechanics

A well-known hallmark of cancer is increased extracellular matrix stiffness in the tumor. This increase in stiffness of the extracellular matrix of tumors is believed to be a driver of metastasis and a regulator of cancer cell behavior via mechano-transduction pathways.





Letting biology take the lead

Unlike generic bioinks, which only act to support cells against the forces of gravity, TTB-series bioinks have been tuned to match the mechanical stiffness and elastic modulus of tumors at that organ site. This allows TTB models to more faithfully replicate the tumor microenvironment.

Case Study Findings 4

The power of tissue-specificity

4.1. Morphological and Invasion Fidelity

Cancer cells embedded in tissue-specific TTB bioinks showed significantly higher expression of markers associated with the Epithelial-to-Mesenchymal Transition (EMT). Specifically, the upregulation of Vimentin and the localized expression of Matrix Metalloproteinases (MMPs), such as MMP-9 and MMP-10, were observed. These markers were significantly attenuated in generic or synthetic scaffolds, suggesting that the TTB-Series bioink is a critical prerequisite for observing metastatic-like behavior in vitro.

4.2. Metabolic and Proliferative Signatures

The metabolic profile of tumor cells in TTB-Series bioinks more closely mirrors in vivo signatures. While generic scaffolds often lead to uncontrolled proliferation (an artifact of the "plastic-like" environment), our tissue-specific matrices uniquely promote a physiological growth rate and the development of nutrient/oxygen gradients, which are essential for testing the efficacy of hypoxia-activated prodrugs or metabolic inhibitors.

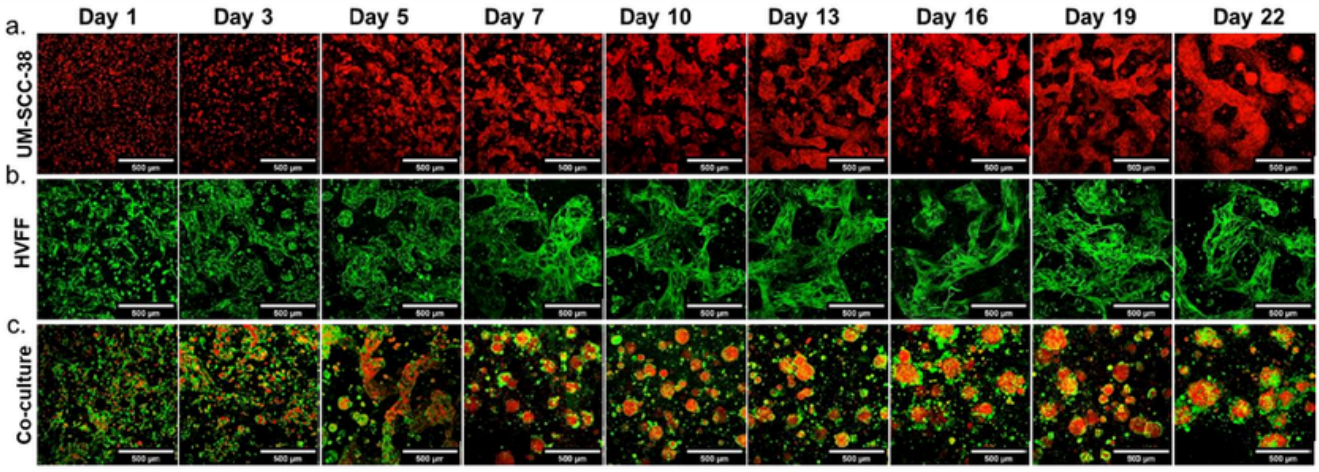


Fig. 2: 3D bioprinted cultures of cells encapsulated in Type-T bioink. Cells were encapsulated in Type-T bioink and bioprinted into discs with 5mm diameter and 500µm height. a. Transduced RFP-UM SCC-38. b. HVFFs stained with Calcein-AM c. 2:1 Co-culture of HVFF: RFP-UM-SCC-38. Red: UM-SCC 38, Green: HVFF. Scalebar 500µm. Z-stack maximum intensity projection.

4.3. Stroma-Tumor Reciprocity

Our findings highlight that fibroblasts (specifically Cancer-Associated Fibroblasts, or CAFs) behave differently depending on the matrix. In TTB-Series bioinks, fibroblasts actively remodel the matrix, depositing new collagen and creating the dense, fibrotic "barrier" seen in clinical biopsies. In short, TissueTinker's technology allows drug penetration testing through a realistic biological barrier.

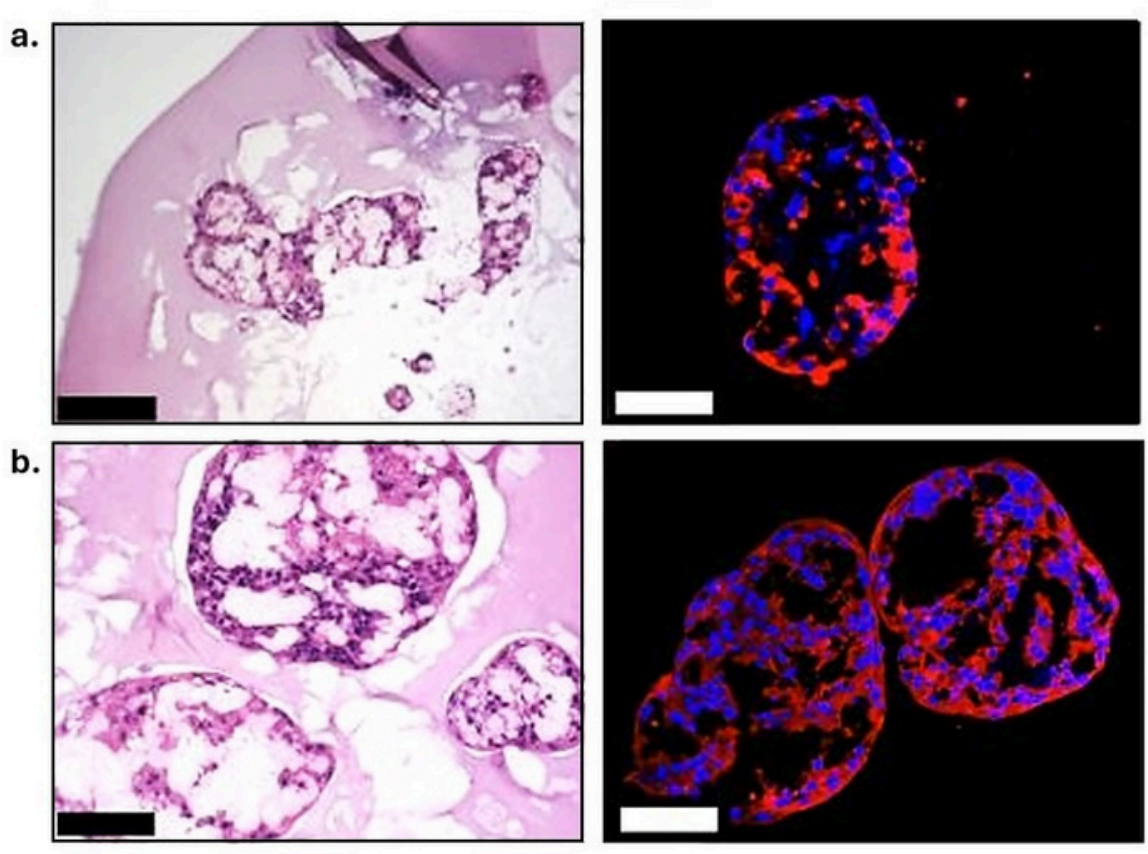


Fig 4. Histological Data of 3D printed cells encapsulated in T-type bioink demonstrating stratified cellular distribution and keratin expression in the bioprinted models. a-b. Hematoxylin and eosin stain in the left column and immunofluorescence stain of polyclonal keratin (red) and DAPI (blue) in the right column. a. UM-SCC-12, and b. UM-SCC-38 cells encapsulated in the bioink blend and bioprinted at 19 days of culture. Scale bar: 100μm

4.4. Translational Perspective and Dosage Performance

TTB-series bioinks uniquely replicate the decrease in chemotherapeutic sensitivity observed in human tissues as reported through IC50 testing. Notably, cell-ECM interactions are responsible for heterogeneous responses that lead to unexpected clinical trial failures. TTB-series bioinks replicate the clinical physiological environment by facilitating cell-cell interactions along with oxygen, nutrients, waste, and drug concentration gradients that create the heterogeneous proliferative and quiescent cell regions that inform the success of various treatments.

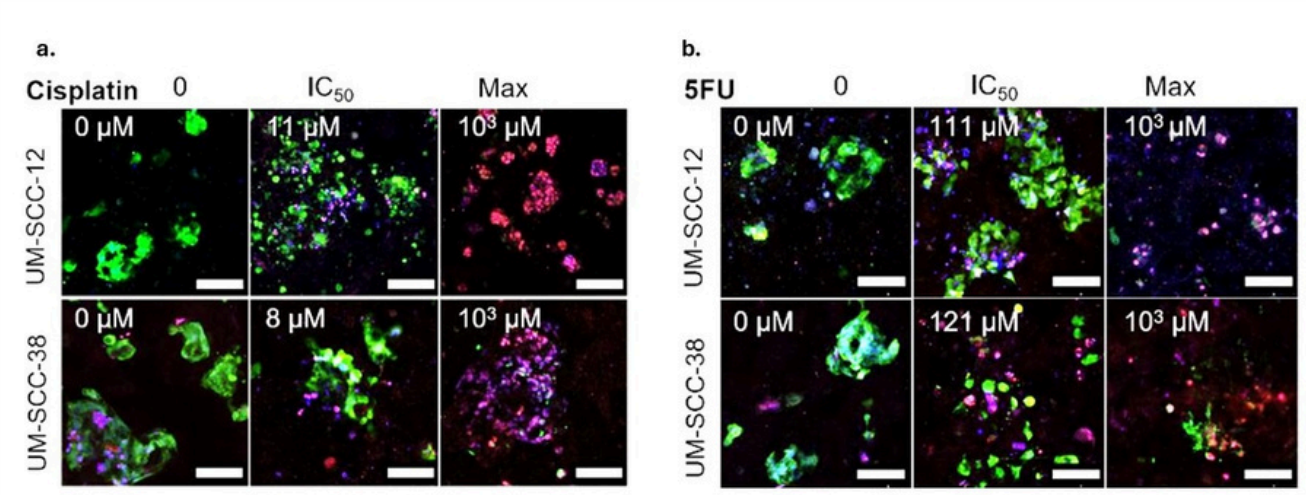
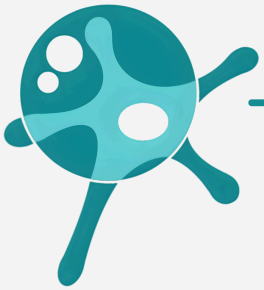


Fig 5. Drug response curves of cells exposed to cisplatin and 5-fluorouracil in T-type bioink. a. Cisplatin. b. 5-fluorouracil. Maximum intensity projection of bioprinted 14 day old spheroids following to a 7-day exposure to varying drug doses (0, IC50, 10 mM) stained with Calcein AM: live (green), Ethidium Homodimer-I: dead (red) and Hoechst 33342: DNA (blue). Scale bar: 100μm.

References 5

Mascort, J. K. (2023). Development and Characterization of a Co-culture Model of Head and Neck Cancer Using Bioprintable Decellularized Extracellular Matrix Bioinks. McGill University (Canada).



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