Advances in 3D bioprinting of tissues/organs

FOR REGENERATIVE MEDICINE AND IN VITRO MODELS

https://www.youtube.com/watch?v=64-ujrKUxLc

3D Bio-Printing (3DBP)

- **Tissue/organ shortage is a major medical challenge** due to donor scarcity and patient immune rejections.
- It is sometimes **difficult to predict or mimic the human disease condition in animal models** during preclinical studies.
- **3DBP** is evolving into an unparalleled multidisciplinary technology for **engineering 3D biological tissue** with complex architecture and composition.
- The technology has emerged as a key driver by precise **deposition and assembly of biomaterials with patient's/donor cells.**
- This advancement has aided in the successful fabrication of in vitro models, preclinical implants, and tissue/organs-like structures.

1. Introduction - Point we will discuss

Current state of 3D-bioprinting strategies for regenerative therapy in organ systems.

Application of 3D bioprinting to fabricated in vitro models to study cancer, infection, drug testing, and safety assessment.

In situ 3D bioprinting: the direct printing of tissues at the injury or defect site for reparative and regenerative therapy.

Issues such as **scalability, immune response, and regulatory** approval.

Clinical trials using 3D printing.

1. Introduction - Tissue engineering and regenerative medicine

Three needs of tissue engineering:

- Maximize organ availability
- Limiting immune response to save lives
- Improving in vitro models

Challenges:

- Replicating organ complexity and revascularization of implanted tissue/organ at the human scale
- Mimic the biologic and functional hierarchy of the native tissues/organs in order to meet specific needs for regenerative therapy

2. Three-dimensional bioprinting (3DBP)

What is it?

3DBP is defined as the utilization of 3DP technology to mimic natural tissue/organ characteristics.

It incorporates a wide range of biomaterials and micro-architecture designs to engineer constructs with desired physical, mechanical, and biological properties.

Most 3DBP technologies for constructing tissue/organ are based on single deposition processes like extrusion, inkjet, or laser-assisted printing.

What is it used for?

3DBP constructs, in vitro models, or implants are used for:

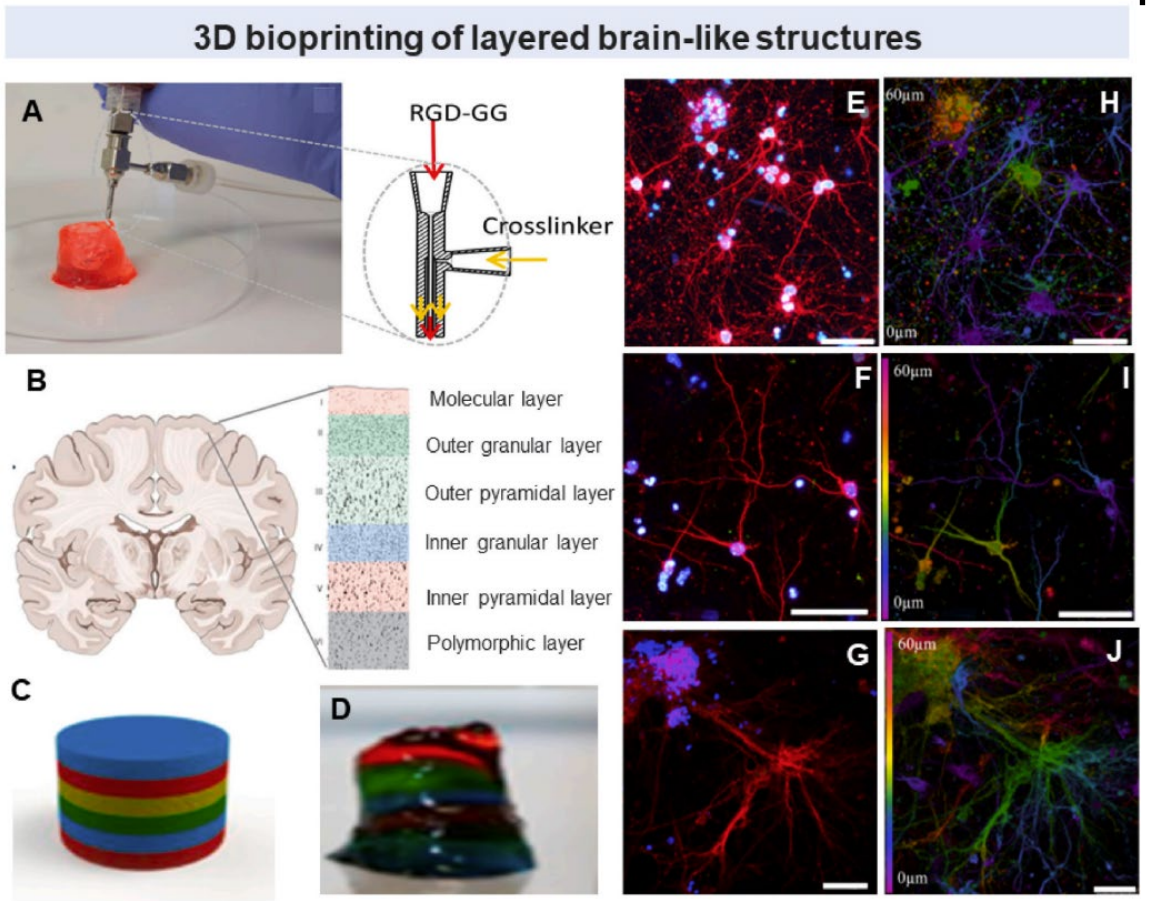
→ replicating the structure and functions of native body tissues **for testing drug, disease, and preclinical therapy effectiveness and replicating the anatomical structure of complex organs.**

In-situ printing is a new frontier in ***highly personalized medicine*** and it is directly implemented in the patient's body.

Three-dimensional bioprinting (3DBP)

What are the steps?	Scanning body part or organ
	Translating a tissue/organ scan into a printable model with software
	Importing the model into slicing software
	Layer-by-layer printing the model with a bioprinter using biomaterial
What are the materials?	Cell-laden hydrogels—also known as bioinks.

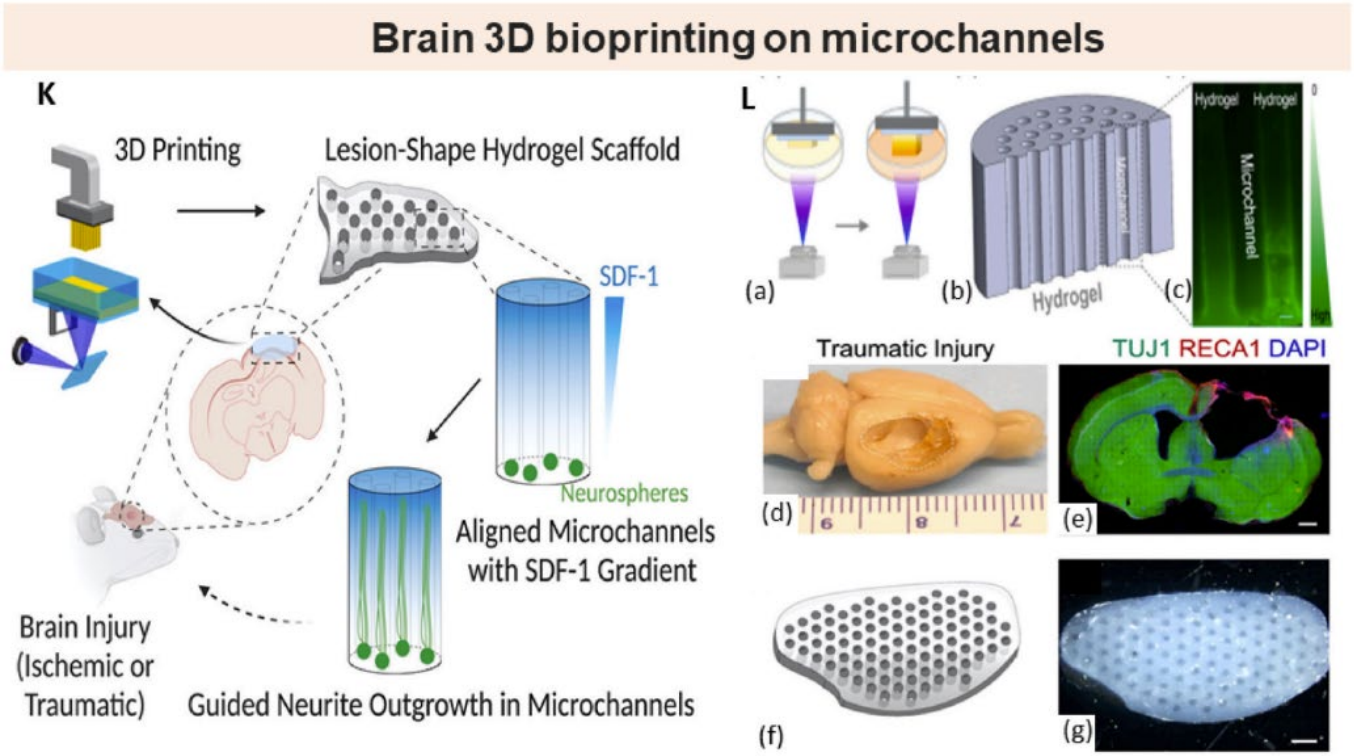
Nervous system: brain and spinal cord



- Bioprinting was used **to develop brain-like multilayer structures** to help neurons grow and establish brain networks.

- These structures help **understand brain functions, injuries, and NDDs** at the tissue or organ levels by mimicking more realistic 3D brain architecture in vitro.

Nervous system: brain and spinal cord

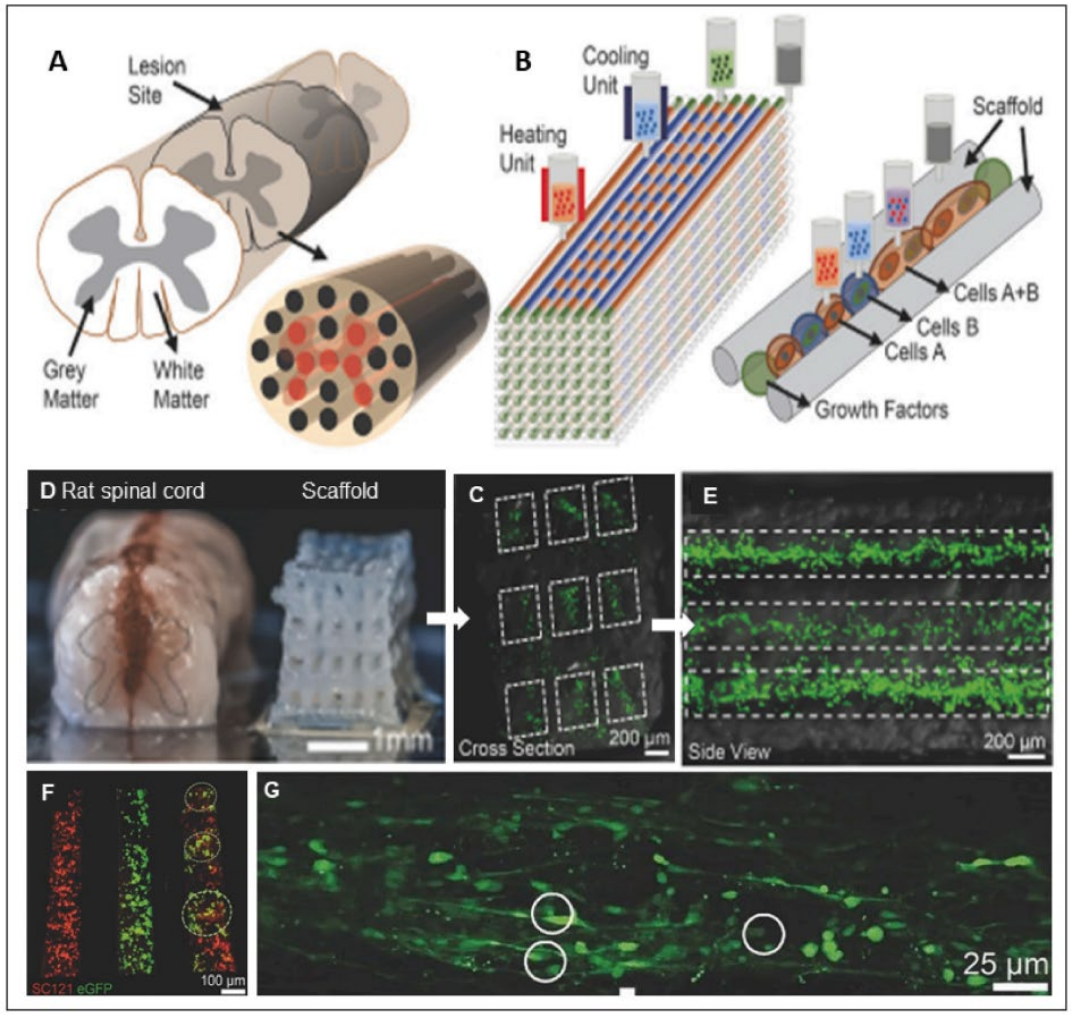


A personalized lesion shape scaffold can be developed using imaging data of injuries.

The hydrogel scaffold was bioprinted for brain injury using microchannels.

The neurospheres get aligned in microchannels and lead to neurite growth.

Nervous system: brain and spinal cord



- For spinal cord injury (SCI), multiple channeled biomimetic scaffolds could provide the **long-term goal of creating personalized clinical implants to treat SCI** patients.

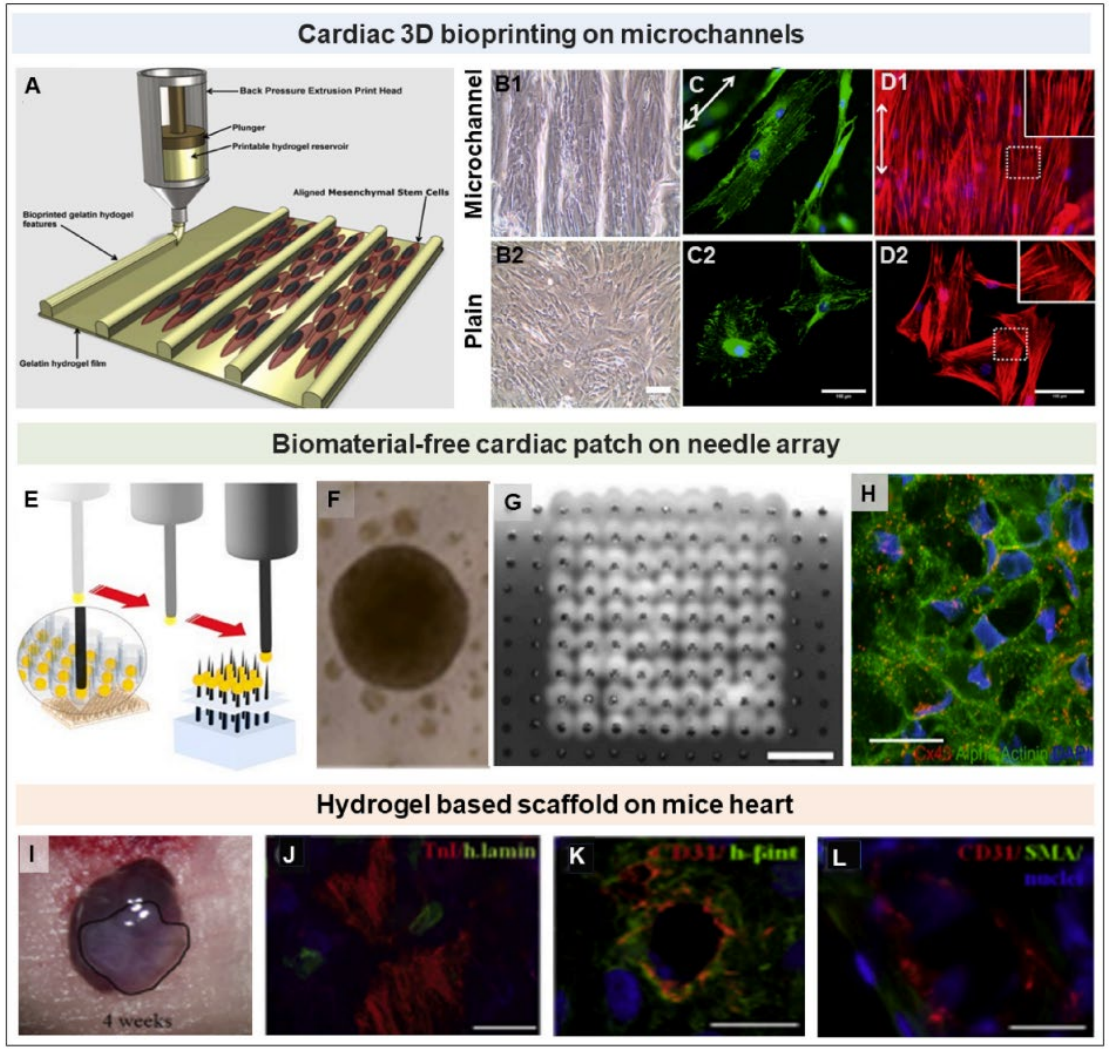
F: sNPCs (neural progenitor cells) (left) OPCs (oligodendrocytes progenitors cells) (middle), and sNPCs and OPCs (right)

G: Calcium imaging of sNPCs with long axon projections and shows cell bodies and adjacent axons.

3DBP for Cardiovascular systems

- **CVDs continue to be the leading cause of death** worldwide, with about 32% (17.9 million) of people dying each year.
- Current **treatments include cell therapy, bypass grafting, implant device, cardiac patches, and organ transplantation.**
- Amongst these, cell therapy has proven successful in regaining muscle functions.
- However, unfortunately, due to the lack of biomimetic ECM, **cells do not survive for a longer period** after injection.
- **3DBP proves to be an attractive and viable option** that can develop scaffolds with natural ECM and stem cell technology for tissue engineering.
- The scaffolds are often composed of biomimetic materials, such as **gelatin, alginate, HA, collagen, fibrinogen**, with/without combinations of crosslinkers or photocurable materials.

Cardiovascular systems

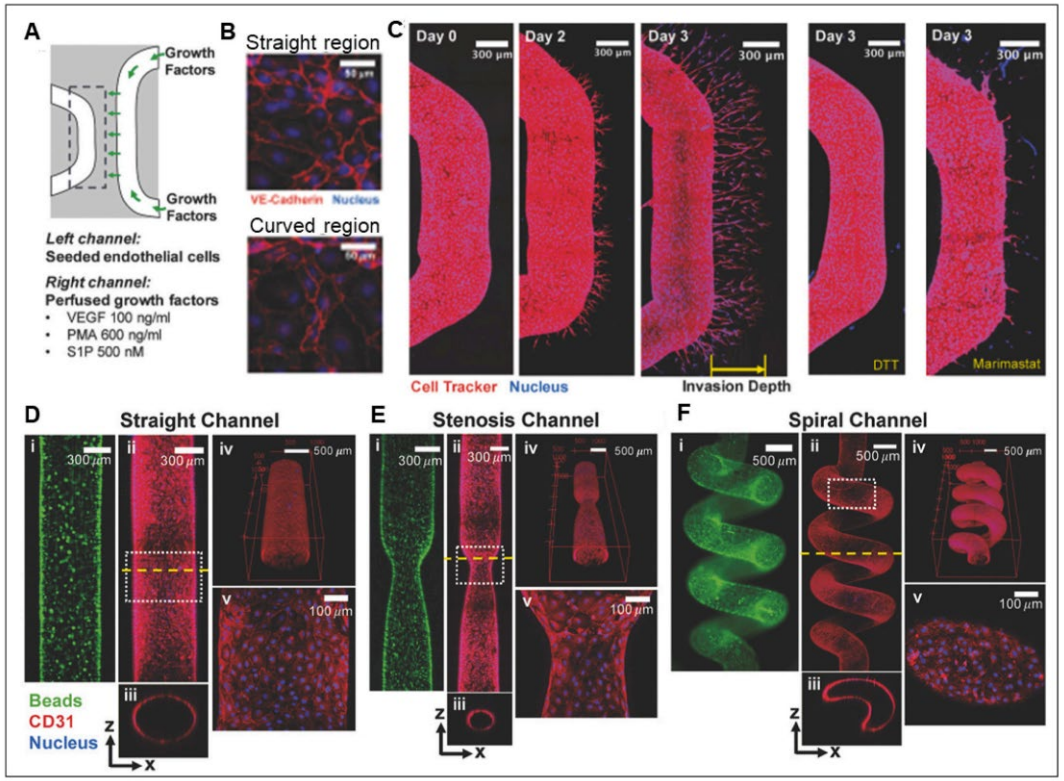


- Microchannel-based 3DBP technique was used to **fabricate a novel cardiac patch using MSCs hydrogels**.
- These channels help in **cellular alignment** and thus **reduce the number of cells needed for cardiac regrowth** and preventing fibrosis.
- Similarly, micro-channeled hydrogel patch was **bio-printed to treat infracted patients**.
- **Multicellular spheroids** were printed onto a needle array to make a tubular construct **to develop a heart tissue**.
- This tissue was used to assess the **therapeutic response, contractile force, and beating rate**.
- In addition, they observed **engraftment and vascularization** post-implantation of the patch.
- Similarly, hydrogel-based scaffold implant showed **tubular, sarcomere, and vessel formation**.

Cardiovascular systems – to consider

- These methods attempted to print a functional patch, but **could not fully mimic the complex microvascular network of the heart**.
- In the future, personalized patient therapy for myocardium regeneration, a **simple scale-up of the printed tissues might not be sufficient.**
- **The choice of cells** that can be expanded to fabricate large tissues is required.
- **Pluripotent stem cells might be a promising alternative to generate cardiovascular patches**.
- **Perfusion, micro-vascularization, avoiding immune reactions** and toxicities are also essential to keep tissue viable after implantation and regeneration.
- Many unknown reasons need to be considered for fully functional heart printing, and there is **still a long road** for cardiac tissue/organ therapy with 3DBP.

Blood vessels



- A **fugitive ink hydrogel deposition onto a support hydrogel** is also used to create microchannels.

- Further, **endothelial cells adhere to these microchannels and form monolayers** that resemble the geometries of blood arteries.

- The **angiogenesis process starts when the cells are exposed to proteases** that help break the hydrogel and start sprouting.

- In addition, the **curvature of channels** also plays a role in angiogenesis.

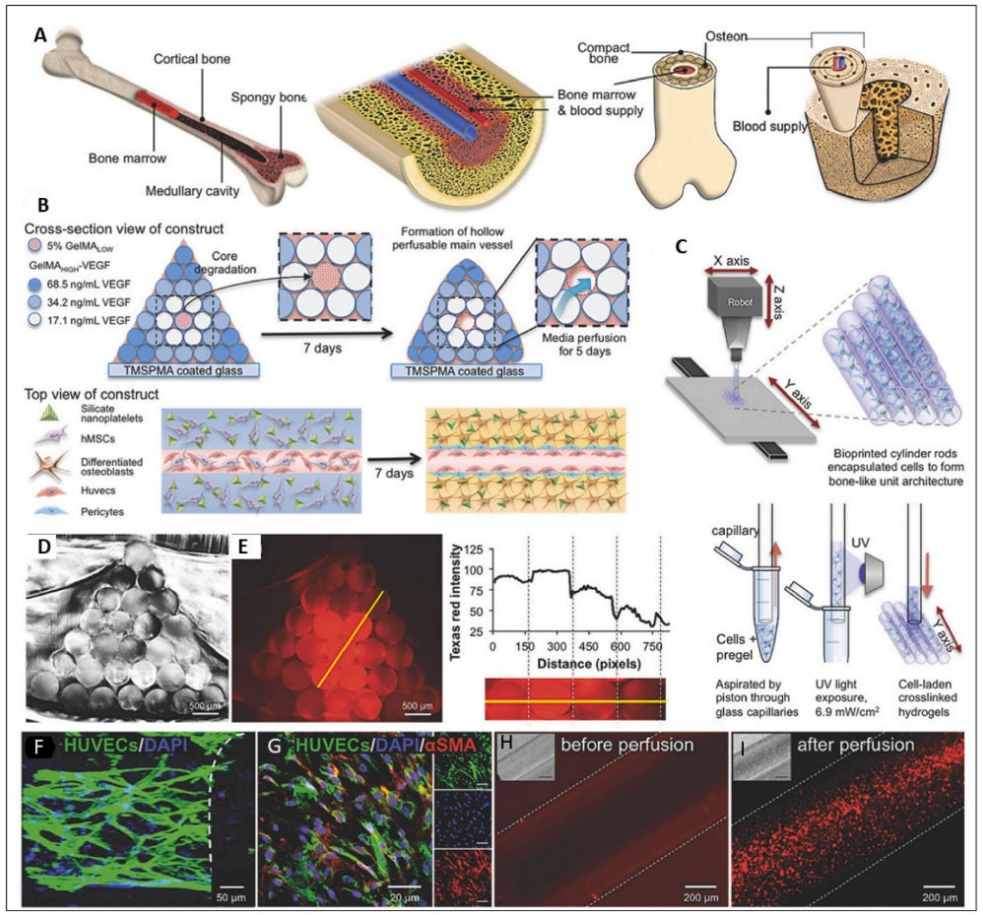
- Further, this method can be used to **enhance or restrict angiogenesis for ischemia or cancer**, respectively.

- However, incorporating these constructs with other tissues, enhancing capillary branching and large-scale engineering is **still challenging.**

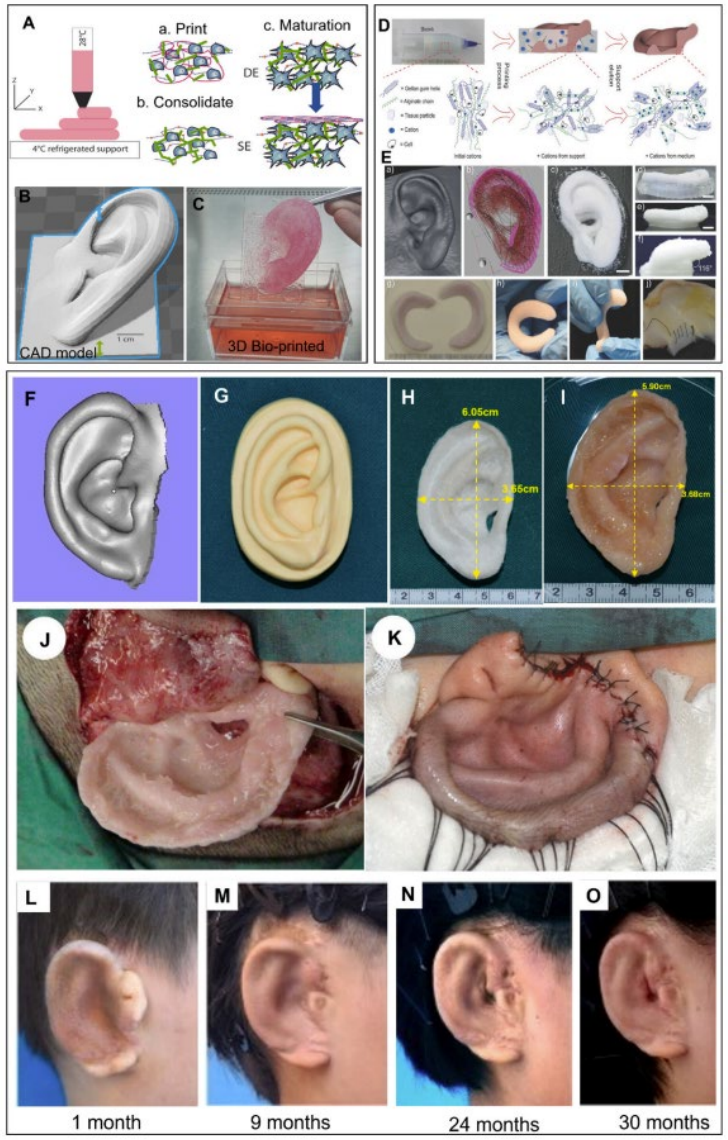
Skeletal system

- According to WHO, osteoporosis causes over **8.9 million fractures/year** worldwide and ranks highest amongst all bone diseases.
- Globally, over 500,000 procedures are operated on annually and are **the highest transplanted tissue after blood.**
- If not treated early, this might seriously affect patients' life quality, leading to bedridden with severe complications.
- The current treatment options available are autografts, allografts, xenogeneic grafts, or bone transplantation.
- However, these treatments might be the **probable source for transmitting infectious diseases and may deal with immunological rejection.**
- Also, the sources for **grafts are limited, and xenogeneic grafts cannot participate in metabolism.**
- Therefore, the 3D bioprinting technique for bone tissue engineering has been developed to overcome these burdens.

Skeletal system

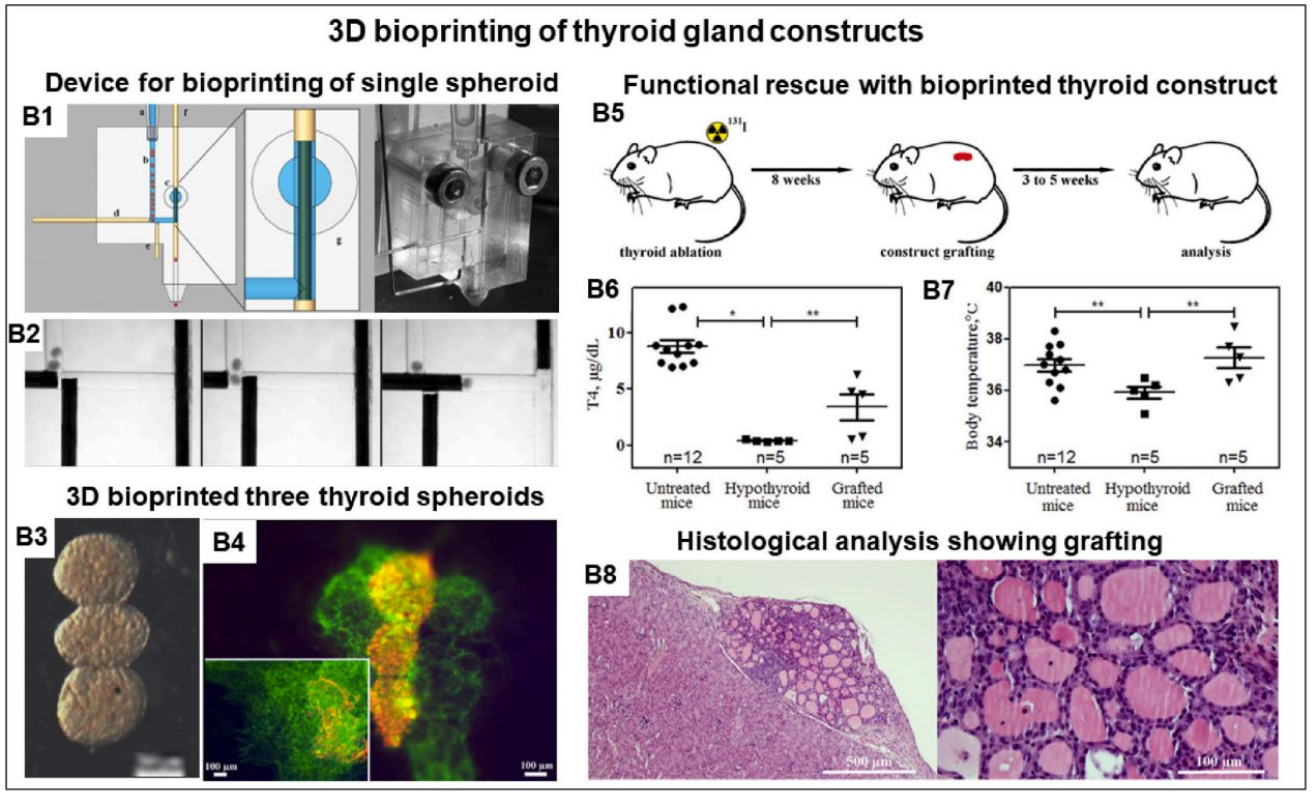


- The 3DBP can **fabricate bones on a large scale** with reproducible custom-tailored tissues and meet the rising need for functionalized bone tissues.
- This technique helps to **bio-print bone scaffolds that can be implanted into humans.**
- The 3D scaffold fabrication **is vital as it provides a template for cell growth, differentiation, mechanical support and forms a hierarchical bone microvascular structure**.
- In addition, an ideal scaffold must have **good biocompatibility, biodegradability, porous structure** (allowing cell infiltration and nutrient transportation), ability to repair the bone defects and functions, and mimic native bone tissue.
- Several biomaterials are used to print these scaffolds, such as **ceramics, cement, natural and synthetic, polymers, polyesters, or metals**.
- There is a wide range of 3DBP techniques, materials in fabricating these scaffolds, and bioprinting complex structures without compromising cell viability.



Cartilage

- Cartilage is an **elastic connective tissue** that forms early in embryogenesis and provides a base for bone growth.
- Some examples of cartilage tissues **are ear, nose, trachea, meniscus, osteochondral, articular cartilage, intervertebral disc, and costal cartilage.**
- **Collagen fibers, proteoglycans, glycosaminoglycans, and high-content water** make up the cartilage matrices, which are viscoelastic, strong, and stiff, and serve to retain and support the body.
- Cartilage appears to be an easy tissue, as **it lacks blood arteries and nerves.**
- It also **can not self-repair**, and if a cartilage defect occurs, it will eventually lead to degeneration and osteoarthritic changes.
- In the presence of cations, using the 3DBP extrusion technique, the cartilaginous constructs such as the human ear are bioprinted.
- Further, 3D cartilage grafts, such as ear, nose, and spinal disc transplants, were produced as **a proof-of-concept study using patient-specific data.**
- **A successful clinical application for auricular repair using patient-specific ear-shaped cartilage has been reported recently.**



Endocrine and exocrine system

- Regeneration of glands or glandular tissues is a global need since their diseases affect physiological functions.

- A **vascularized thyroid gland was bioprinted** using thyrocytes and epithelial cells.

- This bioprinted construct proved its functionality by **restoring homeostasis after implanting** in hypothyroid mice.

- In addition, this construct also helps in **neuronal development and the secretion of growth hormones**.

3. In situ bioprinting (in-situ BP)

One potential 3DBP application is to print **de novo tissue directly onto the specific wound site or defect in the body.**

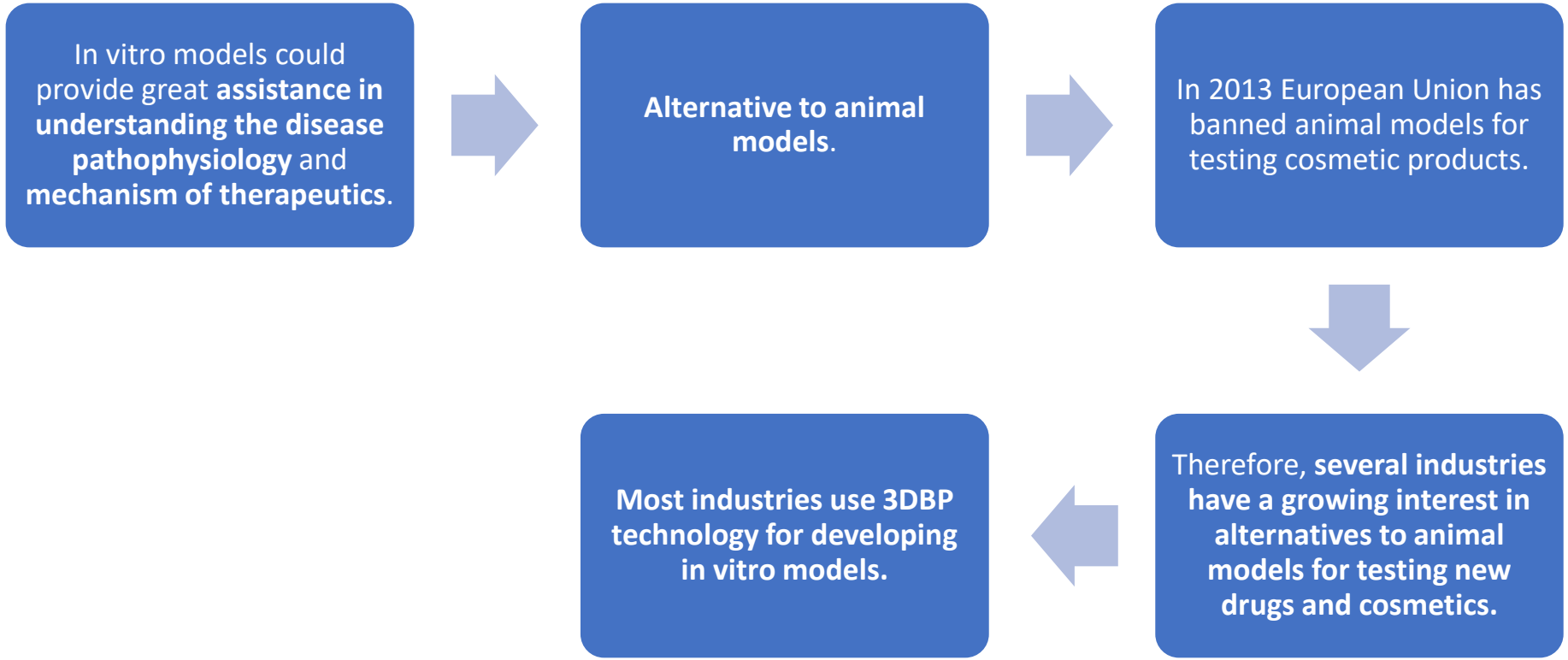
The structure of printed tissue may be tailored to fit into wound/defects with the help of **medical imaging, allowing bioink to be accurately deposited inside the defects**.

It is more favorable than other in vitro BP techniques because the **patient's body is a natural bioreactor providing a natural microenvironmen**t.

On the other hand, **in vitro techniques primarily require the recapitulation of the natural tissue microenvironment to improve cell growth and differentiation**

The in situ technique is promising due to **ease of manipulation, lesser labor involvement, low cost, simplicity, and minimal regulatory impediments**.

4. Bioprinting of in vitro models for biomedical applications

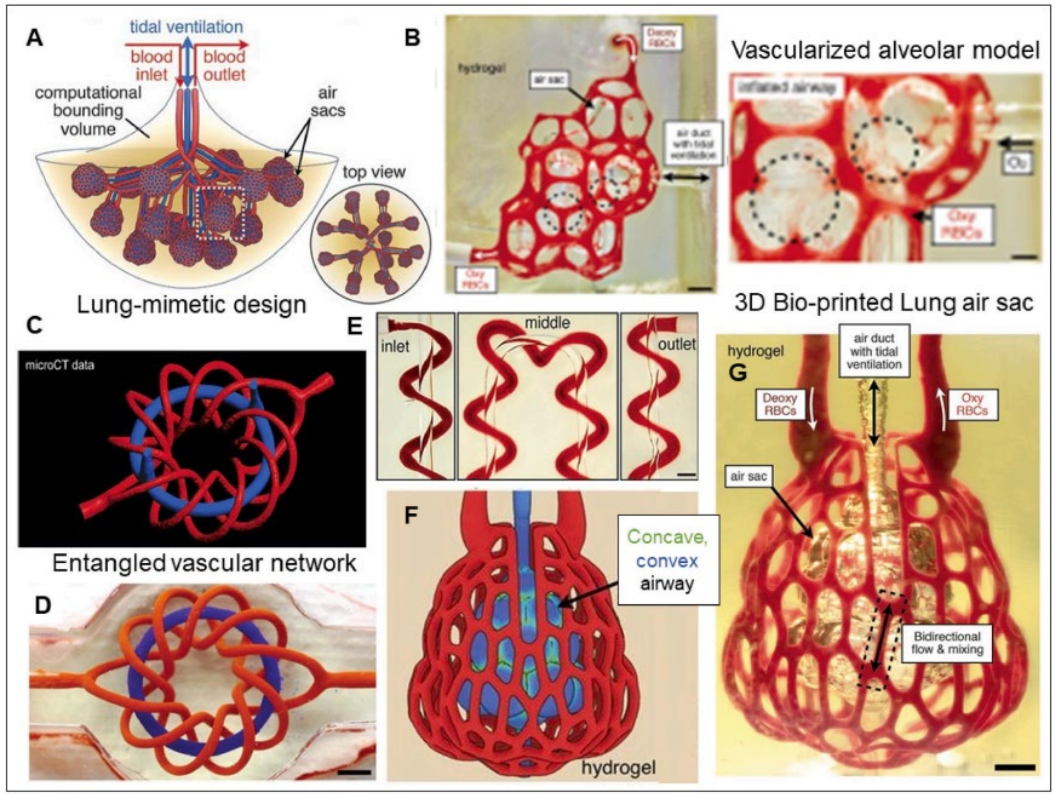


Companies:

1. **Organovo** focuses on developing **3D human tissues** using NovoGen Bioprinter®.
2. **CN Bio and Emulate** companies provided a **liver in vitro model** and toxicity services.
3. **TissUse GmbH** developed an in vitro model platform for **multiple organs** such as skin, intestine, lung, and liver.
4. **Mimetas** is also one of the leading companies in the **3DBP in vitro model** market.

Overall, 3DBP can advance in vitro models engineering for disease modeling, and testing of cosmetics and pharmaceutics.

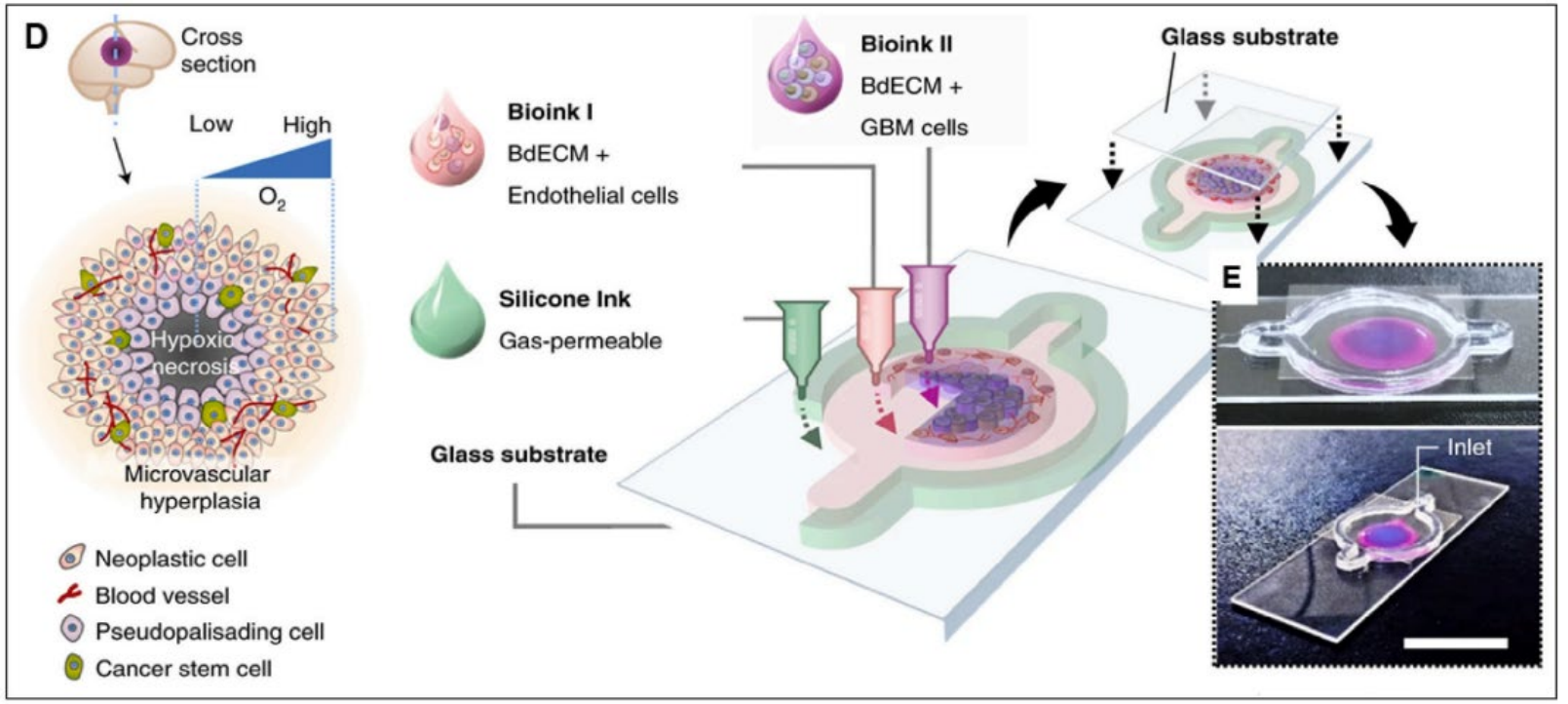
4.1 Organ/tissue models for drug testing, HTS, and safety assessment



- For **high-throughput screening and drug development**, in vitro 3D lung models have been investigated.
- An extrusion-based bioprinter can construct a **human in vitro air–blood barrier model consisting of three layers of endothelial cells, basement membrane, and lung epithelial.**
- The 3DBP process enables the fabrication of thin and **uniform layers of cells, which resemble the physiological function of the native lung**.
- Lung tissue model was fabricated using **silk fibroin**.
- An epithelium-assembled vascular bed system (airway-on-a-chip) was developed, which demonstrated tight junction development and mucus secretion similar to an airway barrier.
- Considering aspects such as **oxygenation, breathing motion, and the flow** of human red blood cells, a more realistic lung model can be constructed.

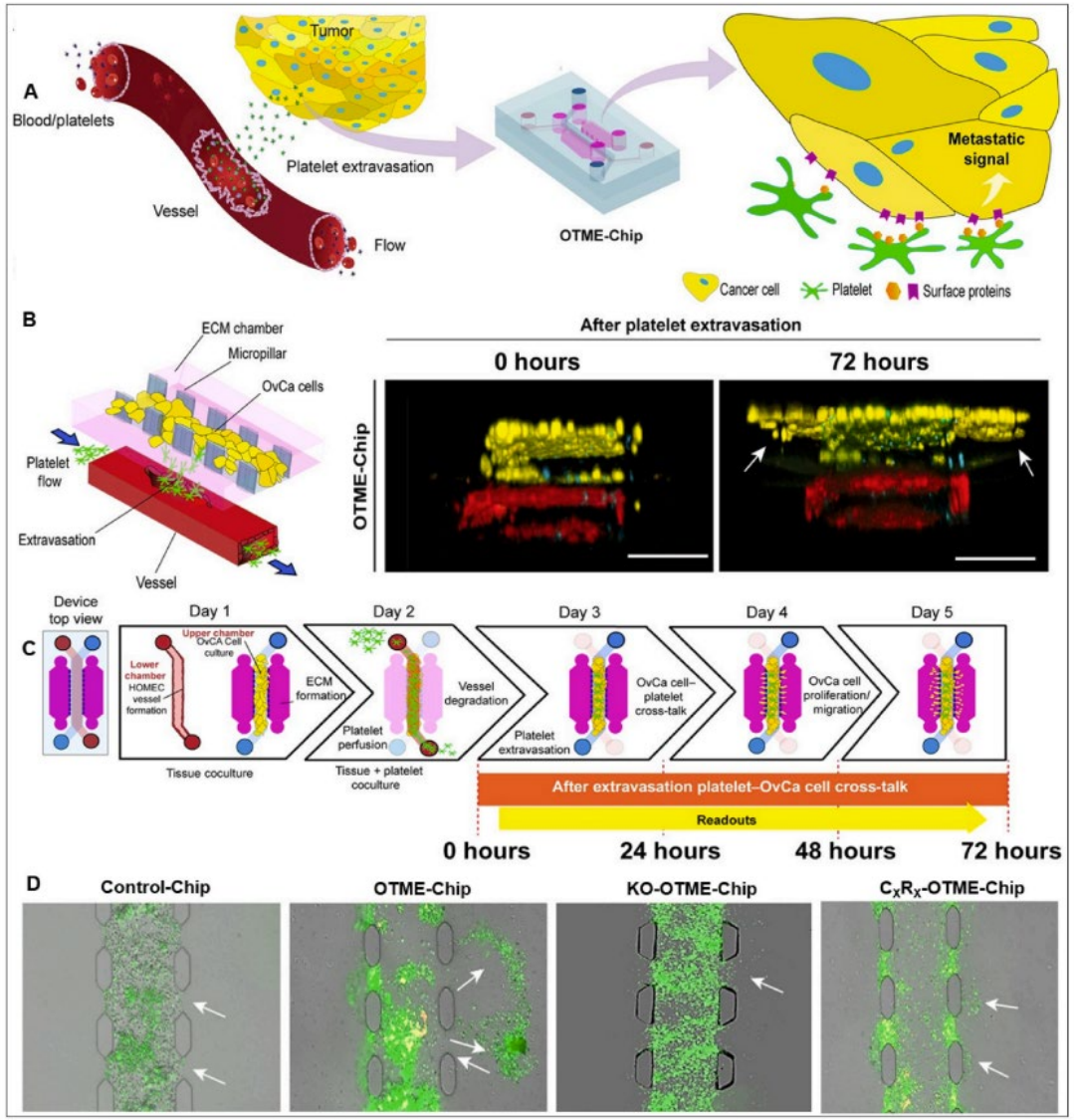
4.2 Cancer models

- According to WHO, cancer is the major cause of death after CVS diseases.
- Despite extensive research, the **survival rate and quality of life are still poor**.
- **Cancer metastasis continues to circumvent mechanistic understanding**, and complex cancer microenvironment is challenging to study in 2D models.
- Thus, **3D cancer models such as scaffolds, spheroids, cancer on-chip** devices are emerging to recapitulate the cancer **microenvironment** closely.
- Further, **3DBP has more advantages over these models as it precisely controls the designing of microstructure and helps incorporate multiple cell types to understand the disease better**.
- Unlike in-vivo models, these **3DBP models are streamlined better to understand the cancer mechanism and screen different chemotherapeutic agents.**



4.2 Cancer models

- Several attempts have been made to bioprint a tumor tissue model having a proper cancer microenvironment.
- **Cancer on-chip models have been developed to improve mimicking of native cancer microenvironment.**
- **Glioblastoma-on-a-chip was developed to examine patient-specific chemoradiotherapy responses**.



4.2 Cancer models

- An ovarian tumor microenvironment (OTME)-Chip was fabricated using a **hydrogel matrix loaded with OVCAR3 cells and assessed the effects of platelet extravasation and antitumor-antiplatelet therapy in ovarian cancer**.

- The 3DBP in vitro microchip in hydrogels can be used to screen various anticancer agent combinations, contributing to the formulation of effective glioblastoma therapy.

- This platform can also be utilized to analyze the **migration behavior of cancerous cells.**

5. Clinical Perspective

- 3D printing technology has drawn the wide attention of inventors due to its **accessibility and fast prototyping ability**.
- During the **COVID-19 pandemic**, 3D printing has enormously helped produce protective gears, medical devices, and isolation ward equipment.
- Despite the several 3D-printed devices, diagnostic tests, and implants under clinical trials , there is **still the scarcity of in vitro tissue/organ models,** which curtails the research and clinical translation of therapies and drugs with high in-vivo efficacy.

Recent approvals:

- Bioprinting of **autologous skin tissue for therapeutic use** (NCT04925323).
- **Colorectal cancer model is engineered for studying chemotherapy response** (NCT04755907).

Those are significant advances that are not yet ready for clinical use. Besides, different organoids, devices, implants are bioengineered to study disease and design personalized medicine.

- Several **3D organoids such as TUMOVASC** (NCT04826913), **GLIOMANOID** (NCT03971812), **ORGANOIDES** (NCT04278326) **are under clinical trials.**
- Additionally, the **National Cancer Institute bioengineered 3D myeloma organoid to study disease biology and drug response** (NCT03890614).

- 3DBP is progressing toward creating opportunities for **developing complex tissues and organs** to have a greater impact on human health.
- Kang and colleagues also demonstrated the **fabrication of vascularized tissues.**
- **Using laser-based 3D printing, scientists reported the development of a bioresorbable tracheal splint to treat tracheomalacia in an infant**.

  FDA approved this intervention for an emergency-use exemption, demonstrating the intervention's critical need.

  The **infant's respiratory symptoms improved significantly**, and successive imaging revealed an open airway.

6. Limitations

- Bioinks have limitations because they must **have unique properties to be optimized for clinical use**.
- Properties such as **structural stability, cell growth promotion, degradation** rate are consistent with tissue regeneration and must be incorporated with cells.
- Presently, bioinks have **been limited in their ability to meet all of these needs**, and a desirable bioink has yet to be identified.
- Another major challenge is **managing a sterile surgical field and time** during the bioprinting process of the constructs.
- These **challenges can be overcome by using a bioreactor platform**, which supports organoid growth and buys time for tissue remodeling.
- In addition, **ethical issues** and challenges is also a hurdle, as fabricating internal tissues/organs may lead to **biosafety and liability concern.**
- Globally, **regulatory authorities found 3DBP challenging and are still unsure how to address this technology's potential** and uncertain risks, such as immune response to bioinks or materials.
- Due to a lack of specific regulations, **FDA refers to CBER guidelines for 3DBP products.**
- The FDA recently approved 3D printed Phonograft, which helps the eardrum heal itself.

7. Summary

- In summary, 3D bioprinting is advancing at a commendable rate but has many challenges that need to be overcome.
- If we overcome these challenges, the rapidly evolving field of 3DBP will provide innovative solutions to engineer tissue/organs, revolutionizing modern medicine and healthcare.

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RESEARCH ARTICLE

Study on drug screening multicellular model for colorectal cancer constructed by three-dimensional bioprinting technology

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Introduction

- 2D cultures are an important model for drug screening for colorectal cancer.
- The 2D culture model is simple, reproducible, and mature.
- The **influence of spatial structure on cells, the interaction between cells, and the interaction between stromal cells and tumor cells** has a great influence on the morphological characteristics and biological functions of tumor cells.
- Sometimes, antitumor drugs with significant inhibitory effects in 2D culture models do not have good pharmacological effects after application in the human body.

- A 3D culture model was developed to improve the success rate of preclinical drug screening.
- Different types of 3D culture models have advantages and limitations.
    - "**sandwich" 3D culture still grows on a flat surface**, tumor cells are stacked layer by layer, no real spatial structure has been established between cells, and cells still lack 3D interaction.
    - **The organoid model is limited by low modeling success rate**, complex process, consumption of various expensive growth factors, and high cost in the culture process.
    - **Patient-derived xenograft (PDX) models have some problems such as ethical disputes,** more time consumption, high cost, and complicated operation.

- At present, suitable **in vitro tumor models for drug screening are urgently needed in clinical practice**, and it is important to develop new drug screening models.

Introduction - 3DBP in colorectal cancer studies

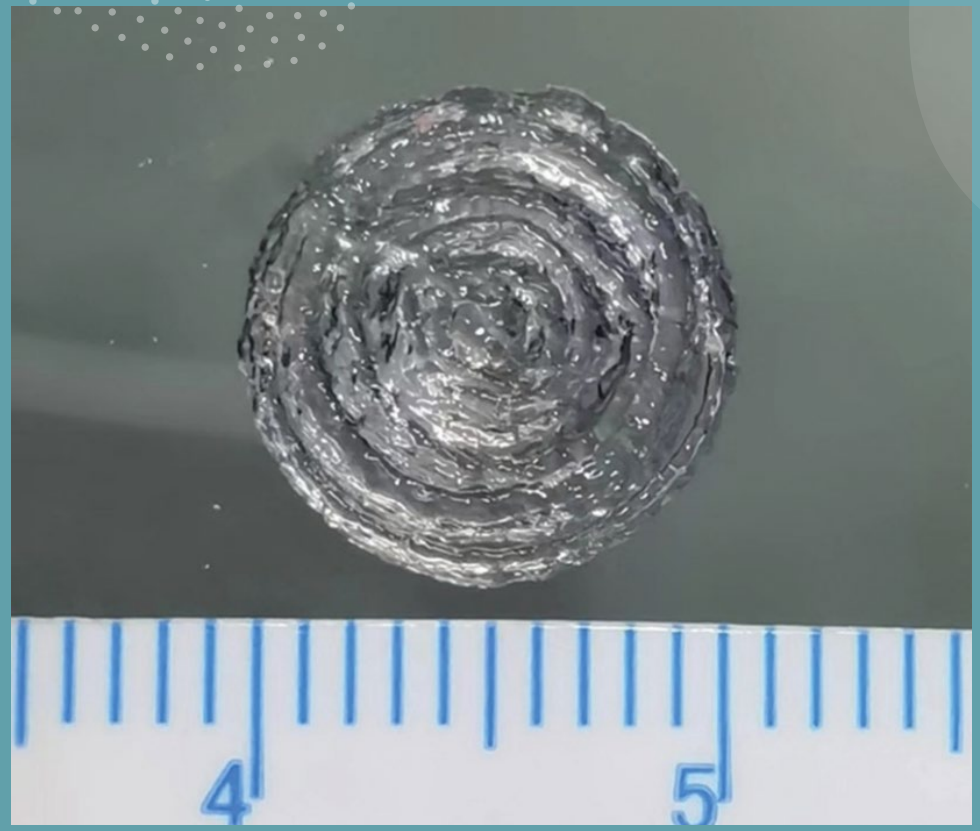
- 3D bioprinting is a convenient, efficient, economical, and easily standardized technology.
- It can use tumor cells as **“cell seeds”** and accurately print according to the model designed by researchers to construct a complex of cells and bio-ink.
- Researchers have used 3D bioprinting technology to explore tumor models .
- However, current research on 3D bioprinting focuses on the optimization of the printing process, the selection of bio-ink, and the optimization of cell viability status.
- There is still **a lack of comprehensive and in-depth biological function** evaluation and drug dose-response experiments of 3D bio-printed tumor models.
- To explore the potential value of the 3D bioprinted model in colorectal cancer drug screening, they constructed a 3D bioprinted single-cell model of colorectal cancer using SW480 cells as colorectal cancer seed cells and gelatin/sodium alginate as bio-ink.
- The 3D bioprinted single-cell model was **compared with the 2D and 3D cultures.**

- They constructed a multicellular model for colorectal cancer drug screening using 3DBP
- They developed a novel tumor cell-stromal cell co-culture model
- They analyzed the potential impact of TME on tumor cells in a 3DBP

Results

Construction of 3D bioprinted multicellular model of colorectal cancer

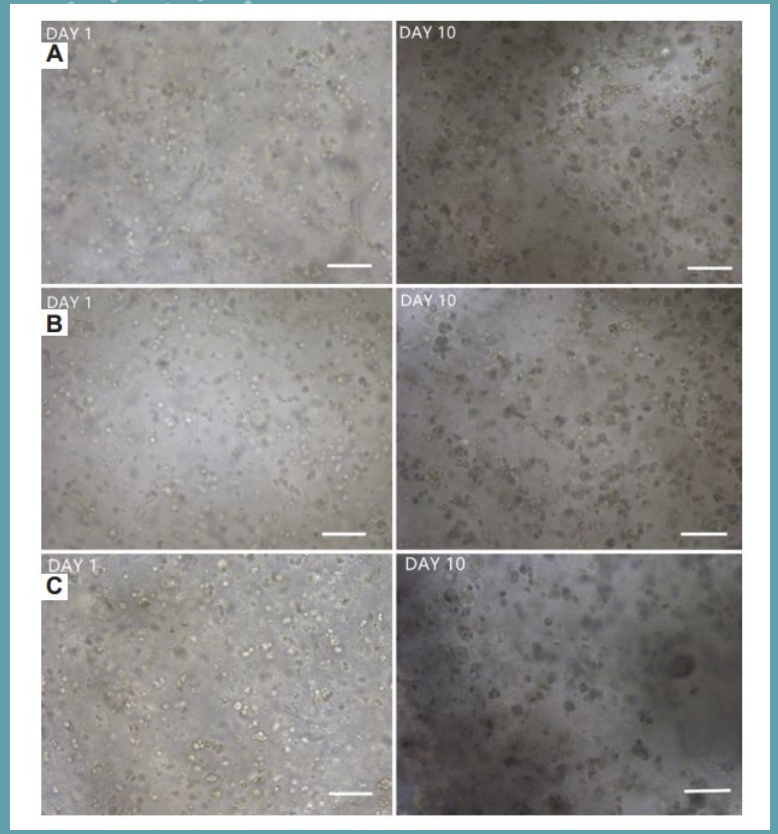
- Concentric axis dual-nozzle 3D bioprinting was used to construct a 3D multicellular model of colorectal cancer in concentric circle model, with **tumors in the inner ring and tumor stromal cells in the outer ring.**
- Extrusion 3D bioprinting is used to construct stable 3D bioprinted models of colorectal cancer **in high throughput.**



Results

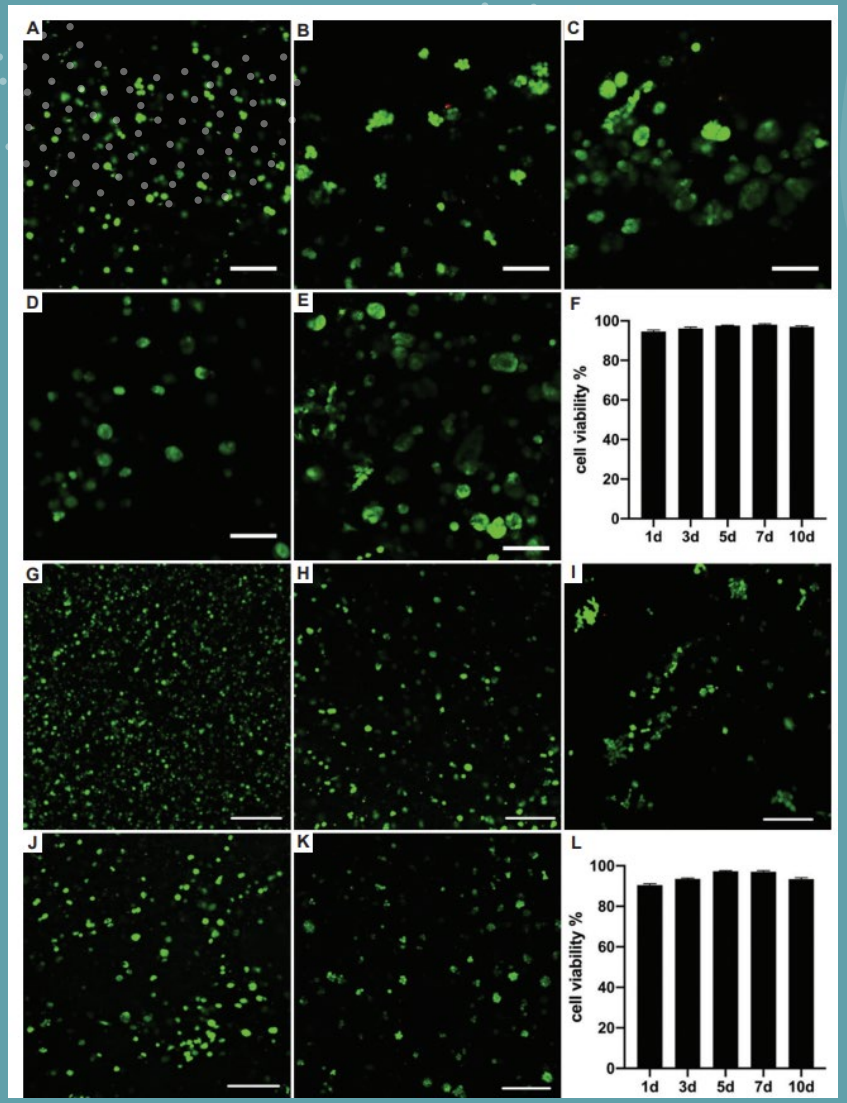
Morphological characteristics of 3D bioprinted colorectal cancer model scattered in the 3D bioprinted colorectal cancer tissues

- Under a low-power light microscope, the 3D bioprinted colorectal cancer model remained **stable from days 1 to 10.**
- **SW480 cells were stable** in the bioprinted tissue under high magnification, and **no tumor cell was observed**.
- From days 1 to 10, the SW480 cells in the 3D bioprinted tissue gathered into clusters, and the SW480 cell clusters became increasingly larger.
- **On the 10th day, dense cell aggregates were scattered in the 3D bioprinted colorectal cancer tissues.**

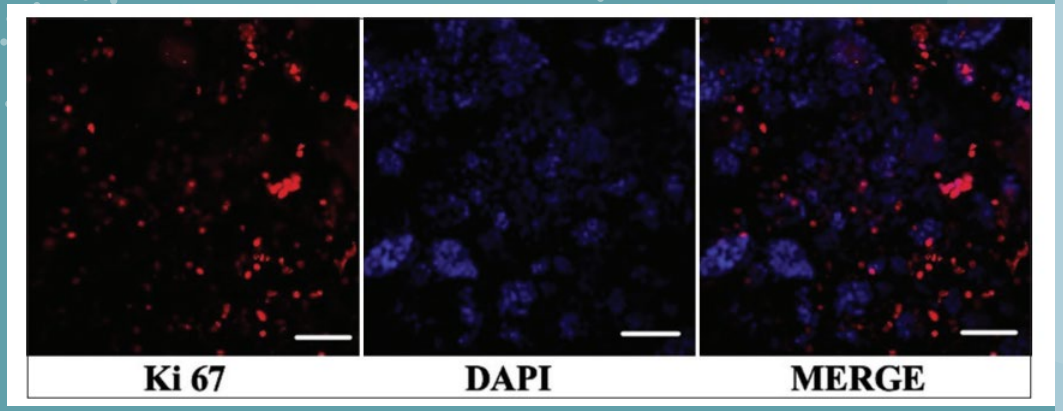


Results
Cell proliferation and survival in 3D bioprinted colorectal cancer model

- The 3D printing models were stained using calcein AM and PI to assess cell survival.
- The survival of SW480 cells was observed using confocal microscopy.
- A high activity of >90% was maintained by day 10.



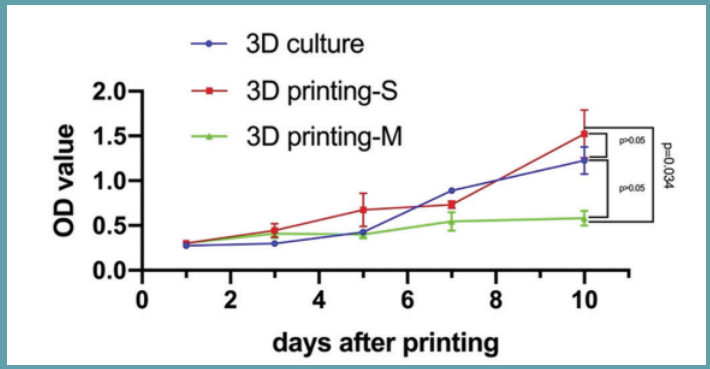
Results
Cell proliferation and survival in 3D bioprinted colorectal cancer model



- Ki67 immunofluorescence was used to observe the proliferation of SW480 cells in the 3D printing on the 7th day, and Ki67 expression was strongly positive in the model.
- **SW480 cells proliferated well in the 3D bioprinted model**.

→ This suggests that the 3D bioprinted model can provide a good environment for the growth of colorectal cancer cells.

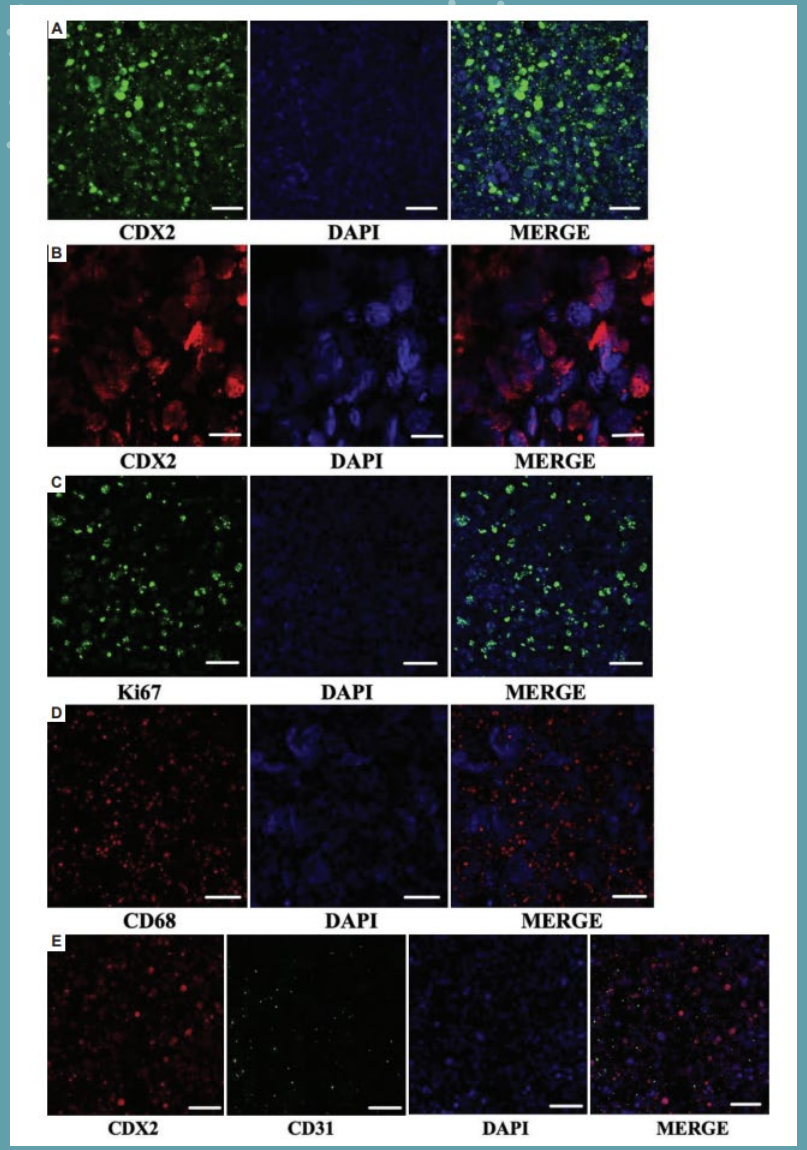
→ CCK-8 was used to detect the proliferation of SW480 CRC cells in the three models. Cell proliferation was measured on days 1, 3, 5, 7, and 10, respectively.



Results
Cell proliferation and survival in 3D bioprinted colorectal cancer model

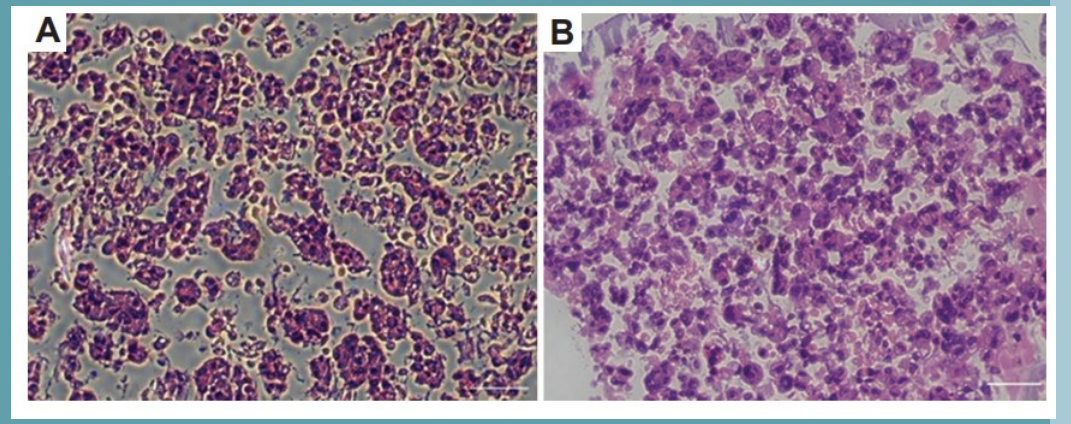
- After 10 days of 3D bioprinting, SW480 cells, macrophage M2, and HUVEC-T/T were stably present in the 3D printing-M.

→ proved that the colorectal cancer multicellular model is stable.



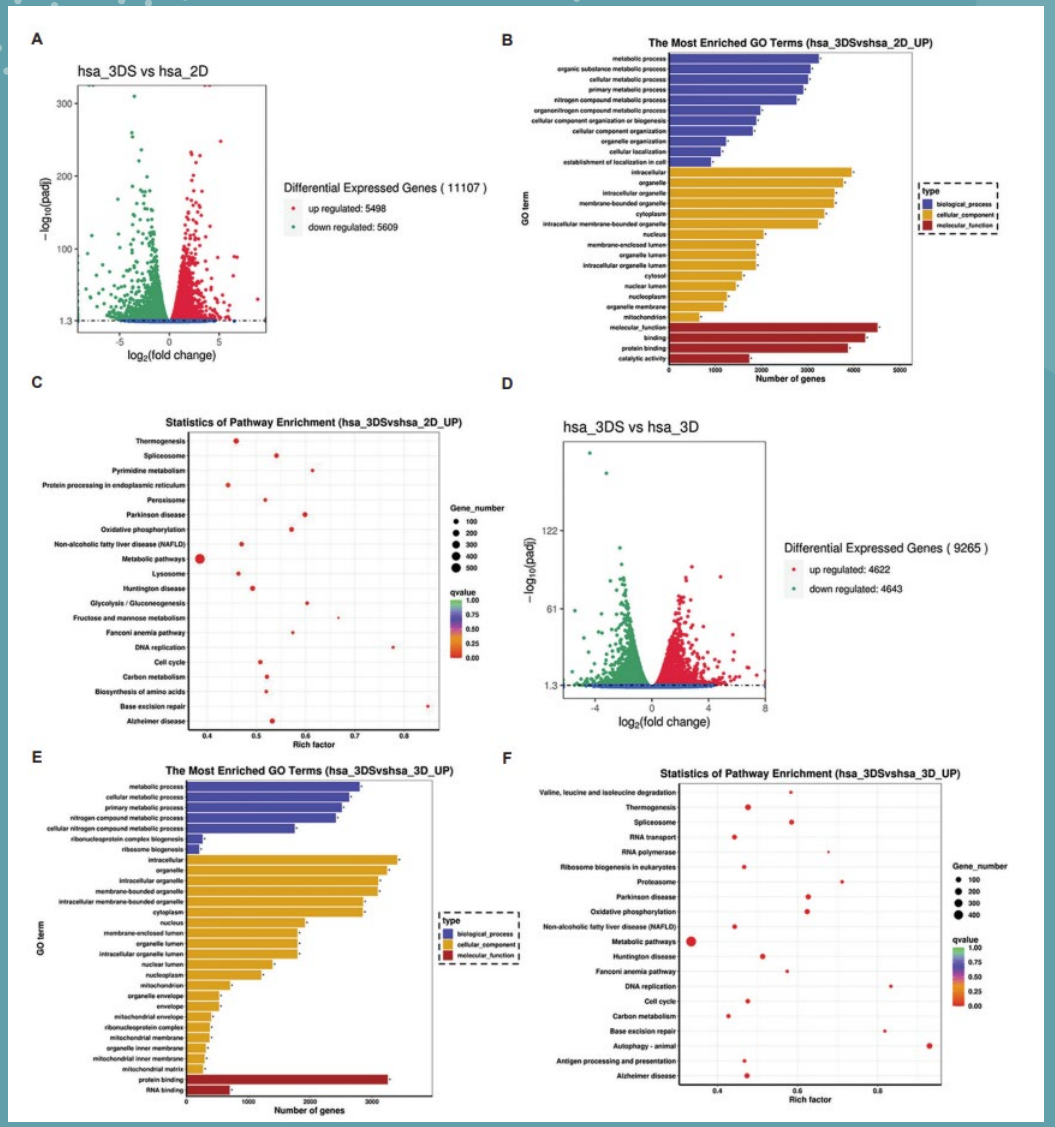
Results
HE staining of frozen sections of 3D bioprinted multicellular colorectal cancer model

- **HE staining** characterized the pathological characteristics of the **tumor cell cluster** in the 3D bio-printed colorectal cancer model.
- Multiple nuclei in the tumor cell cluster could be clearly seen, indicating that it was composed of many cells.
- The pathological characteristics of interstitial cells in the 3D printing-M were observed.



Results Transcriptional profiling of 3D bioprinted colorectal cancer model

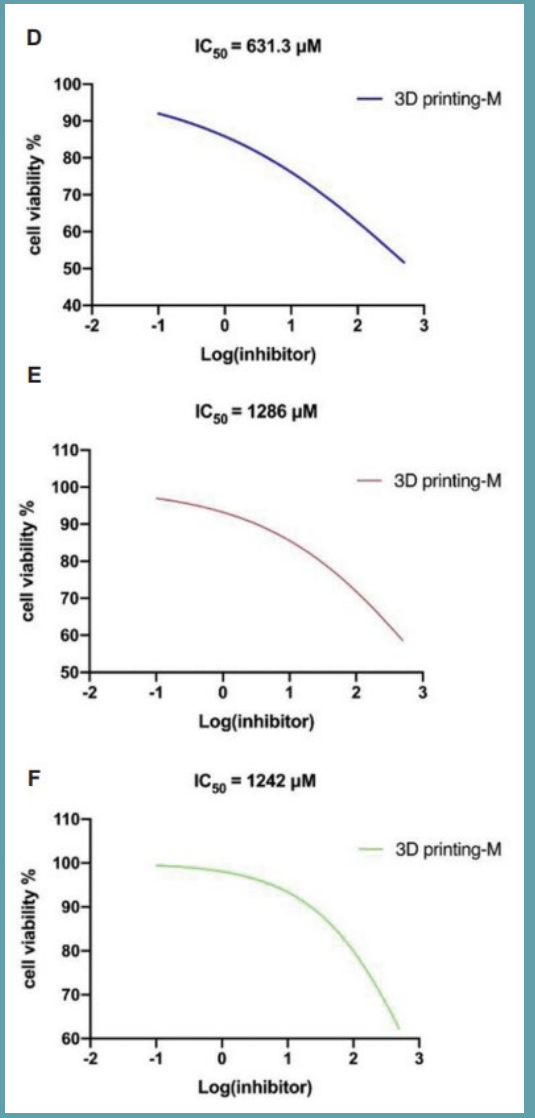
- **Compared to SW480 cells in 2D culture, the gene expression of SW480 cells in the 3D bioprinted model showed significant differences in biological processes, cell composition, and molecular function**.



Results

Effects of antitumor drugs on the 3D printed SW480 model

- **Antitumor drug screening** experiments for the same chemotherapy drugs were performed on the 3D printing-M group, and six concentration gradients.
- The proliferation of tumor cells was detected using CCK8 in the drug screening test.
- The results suggest that the **3D printing-M group was significantly resistant to chemotherapy.**
- The CCK8 value measured at a concentration of 100 μM was similar to the CCK8 value measured at a concentration of 0.1 μm, indicating that **high concentrations of chemotherapy drugs did not significantly inhibit the proliferation of colorectal cancer cells.**
- **Colorectal cancer cells were significantly resistant to chemotherapy in the co-culture system of tumor-associated macrophages M2, endothelial cells, and tumor cells.**



Discussion

- They simulated tumor-related macrophages in the human body using macrophage M2 to better explore the influence of the **TME on chemotherapy resistance**.

  → **3D bioprinted tissue remained more than 95% active** on the 10th day.

  → Immunofluorescence staining of the 3D printing-M showed that **CDX2 of SW480, CD68 of M2 macrophages, and CD31 of endothelial cells were strongly positive on the 10th day.**

  → The results confirmed that **tumor cells, macrophages M2, and endothelial cells could survive well in the 3D printing-M.**

  → They were able to produce **frozen tissue sections of 3D bioprinted models** and understand the morphological characteristics of tumor cells and interstitial cells through HE staining of the frozen sections.

  → The cell lines have a **certain degree of tissue characteristics** depending on the bionic characteristics of the 3D bioprinting technology.

  → KEGG enrichment analysis showed that compared with the 2D culture group, **metabolic pathway, and oxidative phosphorylation pathway were the most significantly upregulated gene enrichment pathways** in the 3D printing-S group.

  → These results suggest that **colorectal cancer cells in the 3D bioprinted model are significantly different from those in the 2D cultures.**

Conclusion

- This is the first time that a research group developed a 3D bioprinted multicellular co-culture model using concentric axis dual-nozzle 3D biopriting.
- Their 3D printing-M was incorporated with SW480 cell lines and was not constructed with primary colorectal cells.
- They considered using more stable cell lines in the modeling stage to ensure the stability and repeatability of the experiment to a certain extent.
- In the future, they plan to validate primary colorectal cancer cells based on this experimental model and plan to use this model for personalized drug prediction.

Thank you for your attention!