REVIEW ARTICLE ON THREE DIMENSIONAL (3D) BIOPRINTING

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Abstract

Three dimensional (3D) bioprinting is the utilization of 3D printing– like techniques to combine cells, growth factors, and/or biomaterials to fabricate biomedical parts, often with the aim of imitating natural tissue characteristics. Generally, 3D bioprinting can utilize a layer-bylayer method to deposit materials known as bioinks to create tissue-like structures that are later used in various medical and tissue engineering fields. 3D bioprinting covers a broad range of bioprinting techniques and biomaterials. Currently, bioprinting can be used to print tissue and organ models to help research drugs and potential treatments. Nonetheless, translation of bioprinted living cellular constructs into clinical application is met with several issues due to the complexity and cell number needed to create functional organs.

3D bioprinting technology along with associated 3D bioprinting strategies including ink-jet printing, extrusion printing, stereolithography and laser assisted bioprinting techniques. Applications of 3D bioprinting technology on construction of various representative tissue and organs, including skin, cardiac, bone and cartilage etc. The steps involved in each of those tissues/organs printing and discuss on the associated technological requirements based on the available reports from recent literature. We finally conclude with current challenges with 3D bioprinting technology along with opportunities for future technological advancement of efficient and cost-effective 3D bioprinting methods.

Keywords

Scaffold, Bioink,

Introduction

3D BIO PRINTING...

3D bio printing is the process of creating cell patterns in a confined space using 3D printing technologies. 3D bio printing is the layer by layer method to deposit materials known as bioinks to create tissue like structure. Currently, bioprinting can be used to print tissues and organs to help research drug and pills.[3] The most latest technology in biofabrication of living structures using tissue engineering is "Bioprinting". Bioprinting is defined as the construction of tissue constructs using a set of techniques that transfer biologically important materials onto a substrate with computer-aided, specialized 3D printers. 3D BIO-PRINTING is the three-dimensional printing of biological tissue and organs through the layering of living cells.[4] It is mainly divided into two :

1. ORGAN TRANSPLANTATION

It refers to transplantation of organs due to organ failure or injury.

2. TISSUE ENGINEERING

It is the study of the growth of new connective tissue, or organs, from cells. Organ failure is a worldwide problem and its only or tissue organ transplantation treatment is replacement.

In vivo fue engineering The Grail Project Focuses on the association of living cells with signaling molecules and supports, known as scaffolds.

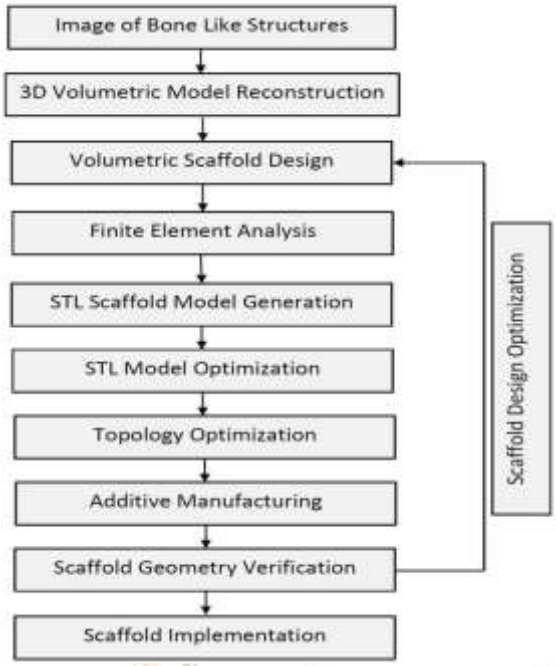
Importance of scaffolds

1. Substrate attachment is required for cell growth and proliferation.

2. Tissue construct must have organ specific shape, the shape of the construct will influence the cell behavior;

3. The scaffold serves not only as an attachment substrate, but also as a source of inductive signals for cell differentiation, migration, proliferation and orientation;

4. The mechanical properties provided initially by the scaffold will be maintained.[8]



Evolution of Tissue Engineering and Bioprinting

1984	**Charles Hull invented Stereolithography**
1996	Dr. Gabor Forgacs observed that cells stick together during embryonic development
2000	Urinary bladder augmentation using a synthetic scaffold seeded with the patients' own cells
2003	Thomas Boland's lab modified an inkjet printer to accommodate and dispense cells in scaffolds

2009	Organovo, creates the NovoGen MMX Bioprinter using Forgacs technology Organovo prints the first human blood vessel without the use of scaffolds
2010	Organavo develops 3D bioprinted disease models made from human cells.
2011	Fundamentals of Tissue Engineering

• Tissue engineering has emerged as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of tissue substitutes.

Examples of engineered grafts: engineered skin, cartilage, bone, blood vessels, skeletal muscle, bladder, trachea and myocardium Three 'classical' tissue- engineering approaches include (i) use of an instructive environment (ii) delivery of repair cells and/or bioactive factors into the damaged area and (iii) cultivation of cells on a biomaterial scaffold in a culture system.

•Tissue engineering opens several exciting possibilities:

i. to create functional grafts

ii. to study stem cell behavior and developmental processes and

iii. to utilize engineered tissues as models for studies of physiology and disease.[3]

Challenges in scaffold based tissue engineering

i. Poor mechanical properties

ii. Inflammation and mechanical failure

iii. Fibrous tissue formation due to scaffold degradation

iv. Mechanical mismatch with surrounding tissue

v. Reduced cell-cell connection

The success of engineering and fabricating functional living structures will depend on understanding the principles of cellular self-assembly and our ability to employ them.

Self-assembly is the autonomous organization of components, from an initial state into a final pattern or structure without external intervention.. Example: Histogenesis and organogenesis.

Cellular self assembly approaches represent an alternative and offer a complement to scaffold based TE.[2]

Developmental mechanisms of cellular self-assembly

It is through cellular self-assembly that the morphologically featureless zygote evolves into the fully developed organism with its numerous structures of widely varied shapes and forms.

1. Cell sorting
2. Tissue fusion
3. Apparent tissue liquidity

These characteristic morphogenetic mechanisms that are utilized in the biofabrication technology.[10]

1. Cell sorting

Cell sorting is a self-assembly process providing a common mechanism to establish cellular compartments and boundaries between distinct tissues.

Differential adhesion hypothesis (DAH): Cells of different. types adhere to each other with different strengths.

This morphogenetic mechanism is most active during embryonic development.

2. Tissue fusion

Tissue fusion is a self-assembly process in which two or more distinct cell populations make contact and coalesce. The fusion process underlies the formation of numerous structures in the embryo.

3. Apparent tissue liquidity

As sorting and fusion strikingly resemble, respectively, the phase separation and coalescence in liquids, it was proposed that adhesive and motile cell populations have apparent liquid like properties.[8]

ADVANTAGES

- ✓ Artificial organs personalized using patients own cells.
- ✓ No host rejection.
- ✓ Eliminate need for immunosuppressant drugs
- ✓ When after a regular organ transplant.
- ✓ Quick availability of organs.
- ✓ Low cost of production.
- ✓ Replace human tissue by full body transplant.
- ✓ Allows scientists to eliminate the wait list of organ transplants.
- ✓ Higher survival rate of printed cells.
- ✓ Offers high precise resolution

DISADVANTAGES

- ✓ Bio printers are costly.
- ✓ Use of stem cells is still controversial.
- ✓ Cost of using stem cells.
- ✓ Possibly more expensive than regular organ transplant.

HIGHLIGHTS...

- ✓ Bio printing is a powerful technology in tissue engineering and organ fabrication.
- ✓ Bio printing for cancer research is an emerging direction.
- ✓ Bio printing technology is transiting to pharmaceutics and high-throughput screening.[6]

Application

Sector	Applications
Industry	Jigs, fixtures, and end-use parts for aeronautical industry Prototypes and spare parts for automotive industry
Medical	Surgical models for perioperative surgical preparations Dental fixtures, bridges, and crowns Customized patient specific implants and prostheses Living tissue scaffolds for tissue engineering and regenerative medicine
Pharmaceutical	Customized implants for drug delivery Tablets, capsules, and other patient specific dosages
Food	Designing and 3D printing complex shaped cakes, cookies, candies, pizzas, and other desserts
Fashion	Jewelry, clothes, shoes, and other accessories
Household	Plates, cups, spoons, holders, and other common household objects
Miscellaneous	Space: building prototypes and parts in space Chemical industry: fabricating complex molecules and compounds Construction: scale models with intricate architectures

6

TYPES OF BIO PRINTER...

- Ink jet printer
- Laser assisted printer
- Extrusion based printer

INKJET PRINTER:

- fast and large scale product
- Minimize the cost and waste

LASER-ASSIATED PRINTER:

- Provides high resolution printing
- It is expansive than other printers

EXTRUSION BASED PRINTER:

- Prints layer by layer

Methods For the Bioprinters

It involves three steps...

- Pre bio printing
- Bio printing
- Post bio printing

PRE BIO PRINTING:

It is the process of creating a model and choosing the materials. The first steps is to obtain a biopsy of the organ.

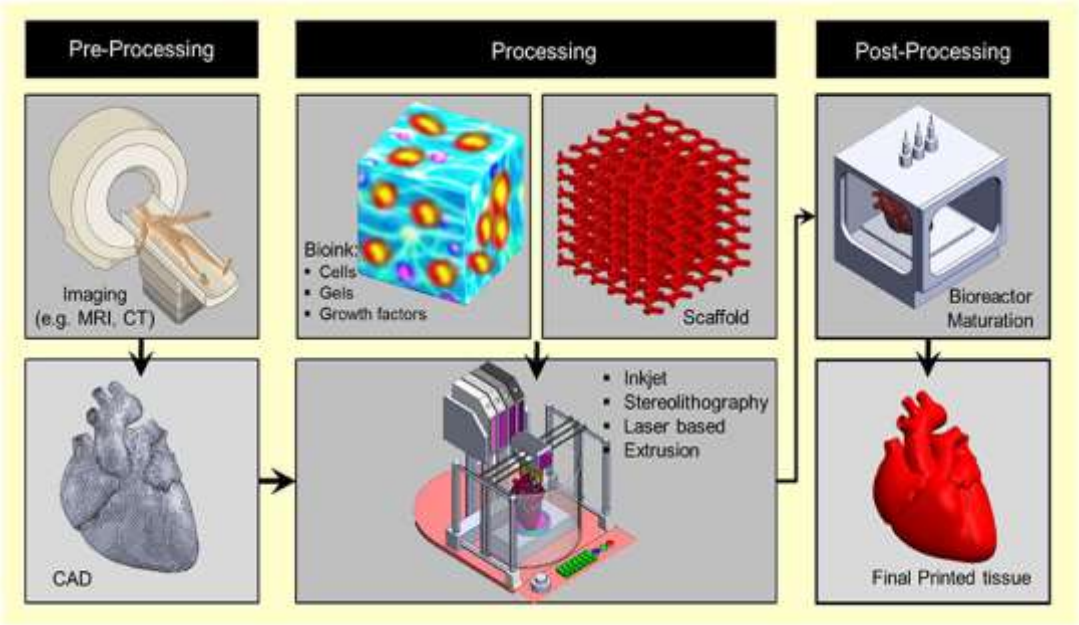
These cells are mixed with a special liquefied material.

BIO PRINTING:

The liquid mixture of cells, known as bio inks are placed in a printer cartridge and deposited using the patients medical scan.

POST BIO PRINTING:

It is the process to create a stable structure from the biological materials.



Method..

3D organ printing (also known as additive manufacturing) is a highly-advanced manufacturing tool that allows for the printing of tissues, and ultimately vital organs, from cells. It is a type of regenerative medicine.

Its history can be traced back to 1983 when the first 3D printer was designed by Charles W. Hull (co-founder of 3D systems).

Structural morphologies Material compositions Physiological functions. Three crucial features before an 3D printed organ could be declared fir for transplantation in humans.

The differences between bio-artificial organs and God- given counterparts must be almost zero in all sense. 3D bioprinting enables the generation of exactly controlled 3D cell models and tissue constructs, by engineering anatomically-shaped substrates with tissue-like complexity.

Due to the high level of control on structure and composition, 3D bioprinting has the potential to solve many critical needs in healthcare research (drug discovery, regenerative medicines functional organ replacement etc.) Customized models of disease can be created using patient- derived stem cells (induced pluripotent stem cells: iPS cells, mesenchymal stem cells).

Depending on the application, a range of materials, methods, and cells can be used to yield the desired tissue construct.

Strategies

Various strategies are there, the crucial feature of this is to build complex organ geometries via spatiotemporal pattern of heterogeneous types of bioinks. These strategies can be classified into 3 groups:

Multi-nozzle rapid prototyping (MNRP) decellularization organ regeneration & combined mold system

What are the ingredients required?

Bioinks

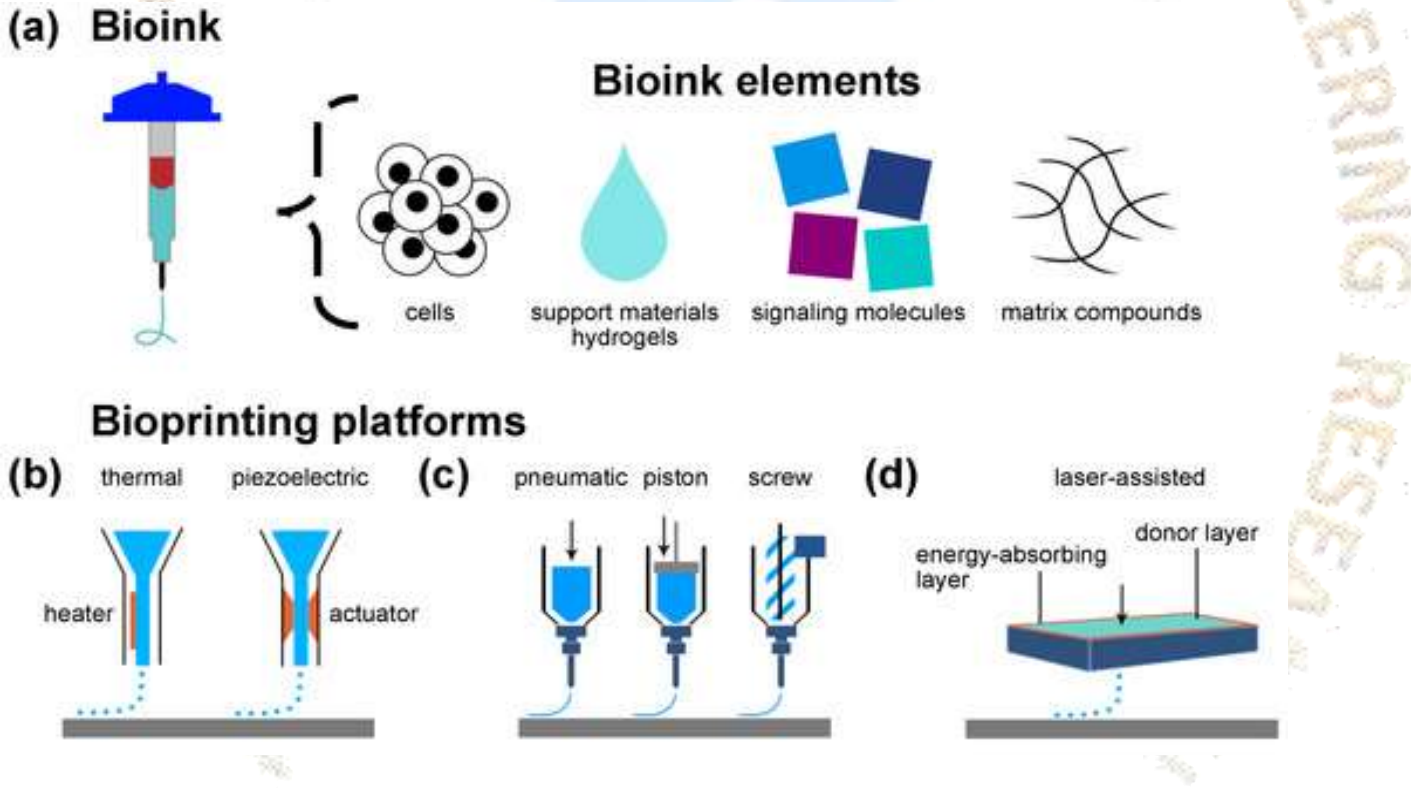
Bioinks comprise living cells and biomaterials that behave like extracellular matrix environment; supporting cell adhesion, proliferation, and differentiation once printing is over.

They must have:

1. Print temperatures which must not exceed physiological temperatures

2. Mild cross-linking conditions.

3. Non-toxic bioactive components which are able to be modified by the cells, once printing is done.



Bioinks for extrusion-based printing (Cell-encapsulating hydrogels)

Cell-encapsulating hydrogels are used in 3D bioprinting to print living tissue structures by forming multicellular bioprinting building blocks.

Cell encapsulation lets exact control over cell attachment and the spatial distribution of the cells and biomolecules within the framework.

Combining multiple cell types and growth factors in a prescribed pattern permits generation of highly-complex tissue paradigms.

Bioprinting materials (for cellular encapsulation) must be biocompatible feature high water content and porosity (for letting encapsulated cells to get nutrients & remove waste.

bio inks	Cell/Tissue Type
Alginate	Chondrocyte, cartilage
Collagen	Hepatocytes
Fibrin	Skeletal Muscle, Neural Tissues
Hyaluronic acid	Fibroblasts, bone cells
Poly (ethylene glycol)-PEG	Fibroblasts, cartilage
Gelatin	Bone, cartilage

Hydrogels

As water-swollen, porous networks, there are perfect materials for tissue engineering, cell-encapsulation & 3D bioprinting.

Hydrogels for 3D bioprinting must also feature adjustable substrate stiffness and allow for network remodeling after printing. This will lead cells to spread, migrate, proliferate & interact.

The most frequently used materials for cell encapsulation are hydrogels (either natural or synthetic).

Natural hydrogels, such as gelatin and collagen, are obtained from animal or human tissues, presenting intrinsic molecular interactions with cells.

Synthetic counterparts like polyethylene glycol are extensively used in bioprinting because of the flexibility of their physical properties.

Depending on the gelation principle, hydrogels can be divided into two categories: physical and chemical hydrogels.

Wide variety of materials are used for bioinks, the most common materials are:

Gelatin methacrylol (GelMA) Collagen

Poly ethylene glycol Pluronic®

Alginate

Decellularized extracellular matrix (ECM)-based materials

Accellular materials (structural scaffolds & polymers) provide structural support for tissue constructs; with bioinks they can generate functional, bioprinted tissues. are porous structures that recapitulate mechanical and biochemical properties of the native extracellular matrix (ECM). Porosity enables cell migration, tissue growth, vascular formation, and cell viability within these structural constructs.

Some common acellular materials are collagen

fibrin chitosan nanocellulose

poly lactic acid polycaprolactone

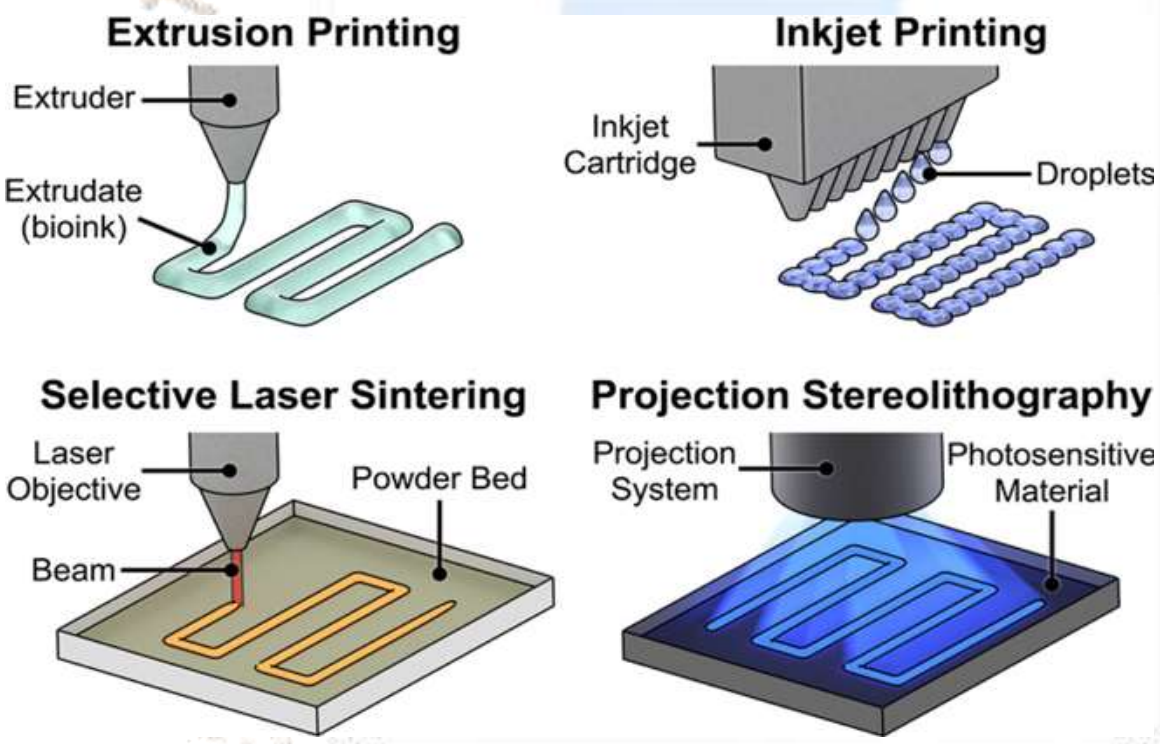
hydroxyapatite B-tricalcium phosphate

What are the machineries required?

Types of 3D bioprinting technologies

Based on the working principles, 5 major types of 3D bioprinting technologies are:

1. Inkjet-based bioprinting
2. Extrusion-based bioprinting
3. Laser-assisted bioprinting
4. Stereolithography-based bioprinting &
5. Microvalve-based bioprinting



Inkjet-based bioprinting

It has been widely used to form 3D cell-laden constructs by constantly ejecting cell-laden droplets on a destination stage by using a thermal or an acoustic actuator.

The nature of the print head designates the 3D construct be built dot by dot for every one layer.

Inkjet based bioprinters are common for bioprinting applications because of their fast printing speed, are compatibility with biological components & low cost.

Clogging should be reduced by adjusting viscosity of printing material.

Laser based stereolithography

SLA involves deflecting a laser beam in a horizontal plane to cure a photosensitive material in order to form a fixed layer. This layer is moved along the vertical axis to allow the next adjoining layer to be created.

This technology is important in the sense that it permits high resolution printing, with a layer thickness as small as 20 μm.

SLS, uses a high powered laser to heat and fuse a powder- based material.

Once each layer is complete, another layer of powder is added to the top of the previous one, to be sintered by the laser to form the next layer.

This is recurrent process, until the whole part is produced.

Fused-deposition modeling

3D printing using FDM involves positioning of an extruding nozzle to deposit strands of material in 3D space.

The extrusion material is thermally melted inside the nozzle, solidifying after cooling upon deposition to create a layer.

Materials in FDM should exhibit a molten phase, making certain polymers and composites well-suited for this process.

Extrusion-based bioprinting

Extrusion-based bioprinting technologies have been extensively used to build cell-laden 3D tissues and organs.

Manufacturing Process

In this a compartment is filled with cell-laden biomaterial.

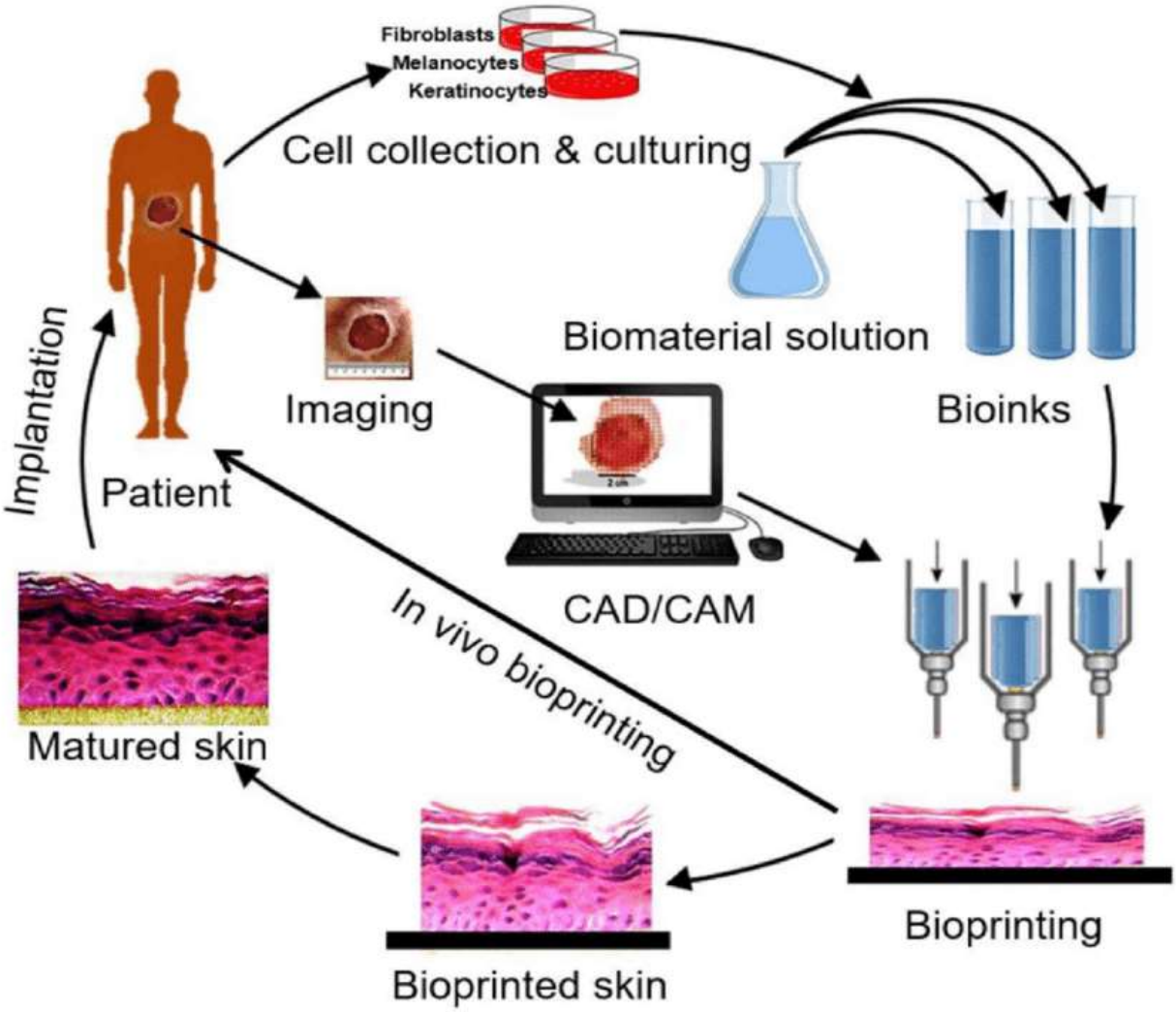
Using either pneumatic or piston-driven extrusion, the material is pushed through the print head.

In order to create cell-laden constructs layer by layer, the print head robotically follows the desired track.

For a physically formed hydrogel, the struts are extruded and gelled on stage upon change in pH, temperature, or other physical condition.

Photo cross linkable ingredients can also be used.

Having extruded a layer of pre-polymer solution, it is ready to be cross-linked by exposure to light.



Step 1

1) IMAGING & DIGITAL

Imaging

APPROACH

For preparing a functional and structural unit of tissue or organ we will need total information from its structure to function at every level from cell to tissue to organ to organ system.

3D view of a tissue can be seen and converted into a data which can be used for bioprinting is prepared by various noninvasive techniques including;

- Computed Tomography(CT)
- X-ray
- Magnetic resonance imaging (MRI)

Step 2

2) DESIGN APPROCH

3D bioprinting is based on 3 central approaches:

1. Biomimicry - To manufacture identical reproductions of cellular and extracellular Biomimicry components of tissue and organ

2. Self-assembly - To replicating biological tissues which is to use embryonic development as a guide.

3. Mini-tissues - To manufacture smaller, functional building blocks which can be fabricated into larger construct by rational design, self assembly or a combination of both

3) MATERIAL SELECTION

The most important part of bioprinting is materials and scaffolds which varies from organ to organ, tissue to tissue and cell to cell Synthetic polymers

• The great accuracy of selection is needed and it must fulfil these criterias:

• Printability

Biocompatibility

• Degradation kinetics and byproducts

Natural polymers

• Structural and mechanical properties

Material biomimicry

Cell selection

4) CELL SOURCES

Different cell types can be used for different uses and purposes which includes:

- Differentiated cells
- Embryonic stem cells or Pluripotent stem cells
- Adult stem cells or Multipotent stem cells Any of these types of cell should have long-term viability & functionality They must divide into sufficient concentration

5) BIOPRINTING

The actual technology, machinery for 3D bioprinting

It is done by three methods:

1) Inkjet Bioprinting

2) Microextrusion

3) Laser-assisted bioprinting(LAB)

THE ENTIRE PROCESS

Medical imaging (CT, MRI)

3D CAD model

Visualized motion program

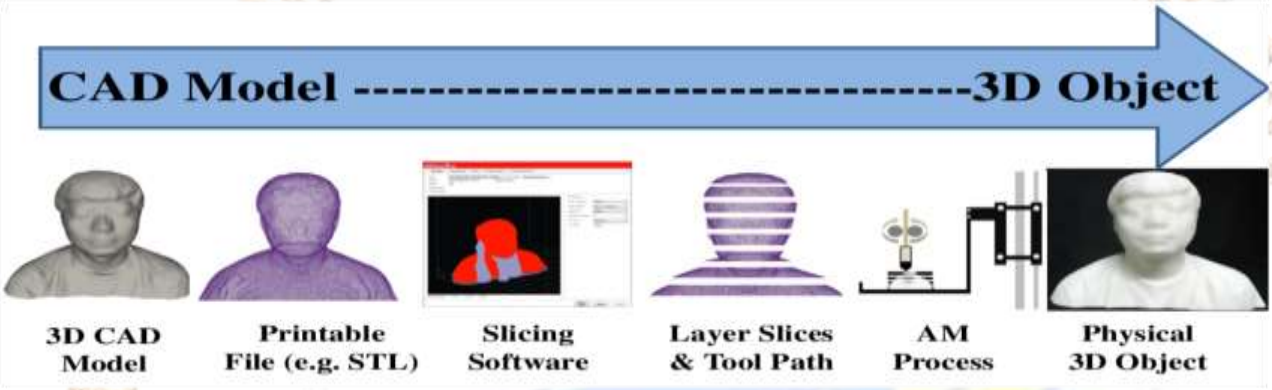
3D printing process

3D bioprinted tissue product

A 3D bioprinting system to produce human-scale tissue constructs with structural integrity(2) The entire process of manufacturing of ear by 3D bioprinting by Prof. Hyun-Wook Kang in his lab at UNIST.

nology research and advisory company [Gartner 2015].

General 3D Printing Process



Technique	Advantage	Disadvantage
Stereolithography-Based	✓ High resolution ✓ Easy to remove trapped materials	✓ Expensive equipment ✓ Only photopolymer materials
Extrusion-Based	✓ Wide range of material choice ✓ Low cost ✓ Good mechanical properties	✓ Limited materials to thermoplastics ✓ Filament required ✓ Viscosity and temperature of materials
Laser-Based	✓ Wide range of material choice ✓ High resolution	✓ Expensive equipment ✓ Heat effects
Inkjet-Based	✓ Low heat effect ✓ High resolution	✓ limited choice of materials ✓ Limited height ✓ Difficulties in Complex 3D geometries ✓ Poor mechanical properties

What are the opportunities & challenges?

Opportunities

- Numerous bioprinting techniques have provided a fully automatic and progressive platform to deposit multiple cell types and ECM like biomaterials to simulate the natural organs; a process that is lacking in traditional tissue-engineering approaches.
- There is still a long way to go to make the large bio-artificial organs to be functional in clinical trials.
- Lakhs of people in the world are waiting for donors of various organs. There is no sure shot guarantee of survival of recipients, they may have to be on immunomodulators for rest of their lives. Situation worsens if patients are victim of more than 1 major diseases. In such cases 3D organ printing will prove a miracle.
- This could open a new world of potentials for the entire medical field, while directly helping patients who need replacement organs.
- Instead of waiting for an appropriate donor or having the risk of their body rejecting a transplanted organ, 3D printed organs will allow patients to have a tailored organ designed specifically to replace their faulty ones.
- Just by looking at models, doctors can avoid potential complications that could have been unexpected without the physical model.

These type of models can be used for training and better understanding.

Challenges

- Major obstacles vascularization and 3D anatomically-relevant biological structures
- Still in embryonic stage as lots of Ifs & Buts (related to methodology, reproducibility and other technical know-hows) besides regulatory issues like ethics etc.
- Currently available tools and models are perhaps not that much powerful to enable experts to insert this initial prototypes or their refined versions to be inserted (into a potential recipient) with the same efficacy as a normal organ.
- The industry is still lagging behind in the bioprinting of human centered tissues/organs due to the intricacies in tissue-specific extracellular matrices & tissue maturation process, the lack of suitable co-culture medium to support multiple types of cells and the requirement of further tissue conditioning before implantation.
- An organ such as a kidney consists of thirty cell types; it means different arrangements need to be done in machineries as per this varied cell types.
- E. g. fabricating or printing heart tissues and organ models remains a great challenge owing to the hierarchical structure of the native myocardium. The requirement of integrating blood vessels brings added difficulty, restrictive the available approaches that are suitable to produce integrated cardiovascular organoids.
- It is must to overcome the technical challenges in creating tissue-specific bio-inks and improving the tissue maturation process.
- It is hoped that in the future combined multi-nozzle organ 3D bioprinting technologies will offer an unparalleled adaptability and competence in mimicking the natural organs in every manner.

3D BIO PRINTING-MEDICAL FIELD...

- Organs
- Stem cell
- Skin
- Bone and cartilage
- Surgical tools
- Cancer research
- Heart and blood vessels

Current Progress

Ear: 250 um cells and collagen from rat tail make human ear in 15 min. Post-processing 3 months. To serve children with hearing loss due to malformed outer ear.

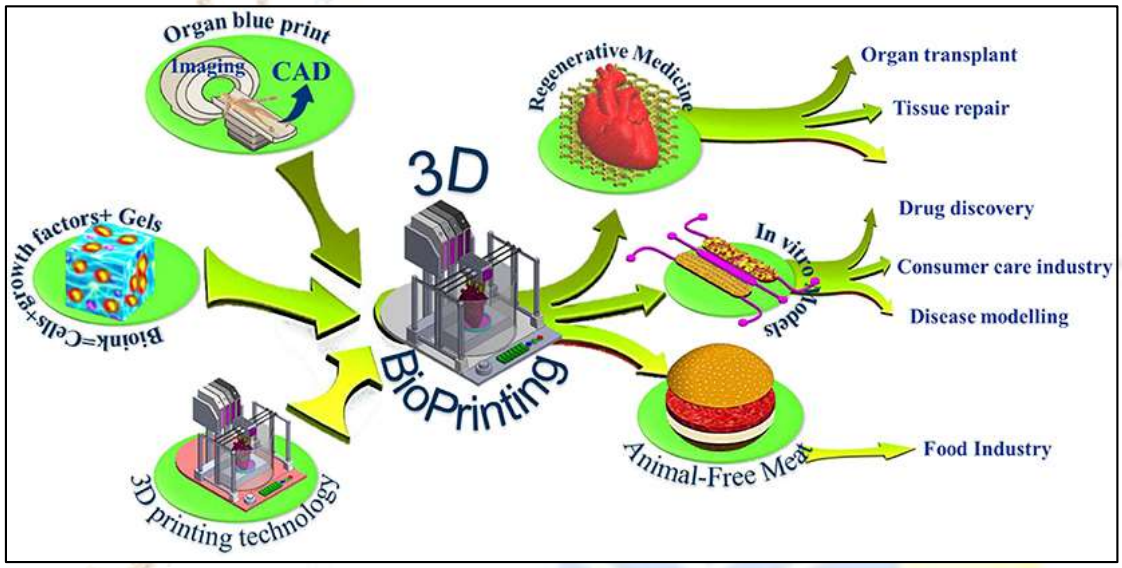
Kidneys: Layer-by-layer building of scaffold and deposition of kidney cells. Assembly to be transplanted into patient. Degradation of scaffold to follow in-vivo.

Blood Vessels: Rigid but non-toxic sugar filaments form core. Cells deposited around filaments. Subsequent blood flow dissolves sugar.

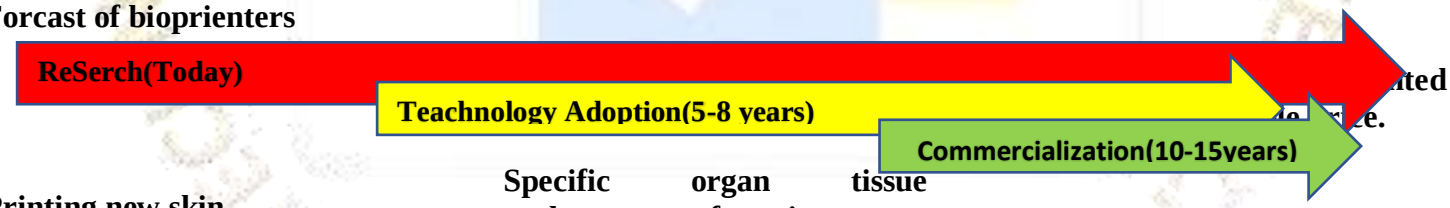
Skin grafts: laser scan wound to determine depth and area. One inkjet ejects enzymes and second, cells. Layer is finally sealed by human skin cells. Useful in war and disaster zones.

Bones: Print scaffold with ceramic or Titanium powder, incubation of 1 day in culture of human stem cells. Repair of complex fractures in accident survivors.

Drug testing: $1.2bn to make a new drug in 12 years. 1 in 5000 has a chance to make it to market. 20-50% drug fail from pre-clinical animal trails to human trials.



Forcast of bioprienters



Printing new skin.

Specific organ tissue replacement for important organs like heart and kidney.

Printing cartilage and bones.

Personalized replacement for 3D printed joints with custom fit.

Liver kidney replacement companies reach maturity.

Printing replacement organs.

Life saving 3D printed organ replacement

3Dprinted replacement for all body organs available.

Pros & Cons

- Artificial organ personalized using patients own cells
- No host rejection
- Eliminate need for immunosuppressant drugs needed after a regular organ transplant
- Eliminate organ donation
- No waiting period

-Bioprinters are costly

- Possibly more expensive than regular organ transplant
- Use of stem cells is still controversial

-Cost of using stem cells

CONCLUSION...

The field is at an early stage, it has already succeeded in creating several tissues at human scale that are approaching the functionality required for transplantation. With the continuous growth of the world's population, and increase of human life expectancy, more cases of organ failure and tissue damage appear. Most common bio printing methods were described and discussed with their characteristics and limitations.In terms of future perspectives for this work, more bio print testing would be needed to be done to optimize the bio ink, substrate and the process parameters.

Additive manufacturing in the context of bioprinting offers a huge potential in the field of tissue and organ regeneration. It enables the fabrication of physiologically-relevant tissue with better and consistent functional outcomes in patients. Such techniques are advantageous over autografting or allografting considering autologous grafts cause unnecessary stress on the patient and there is an acute shortage of allograft donors. 3D bioprinting presents a unique opportunity in that it builds the tissue from bottom up and as such the risk of immunological graft rejection is not present all the while mitigating the issues related to donor scarcity. The use of 3D bioprinting could potentially lead to a personalized treatment for the patient which translates to better clinical outcomes as well as is aesthetically pleasing. However, despite all the advances that have been made in the field, there are still many challenges with regards to the biocompatibility and integration of the printed construct with the body. Maintenance of cell viability in the bio-ink formulation.

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