
The first symposium on bioprinting in tissue engineering
بسم الله الرحمن الرحيم

Bioprinting:

History, Achievements, Challenges and Future

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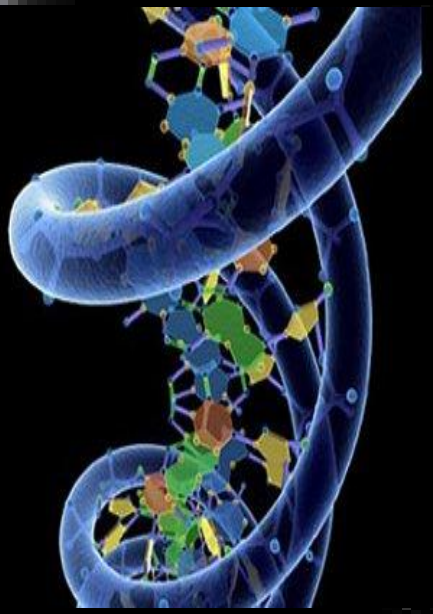
Tissue Engineering as a science and technology

Scientists discover the world that exists;

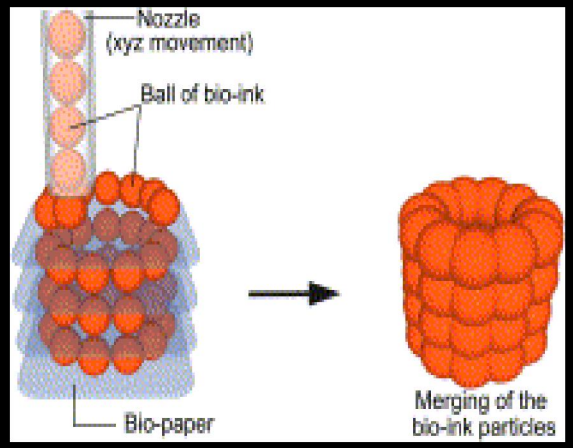
Engineers create the world that never was

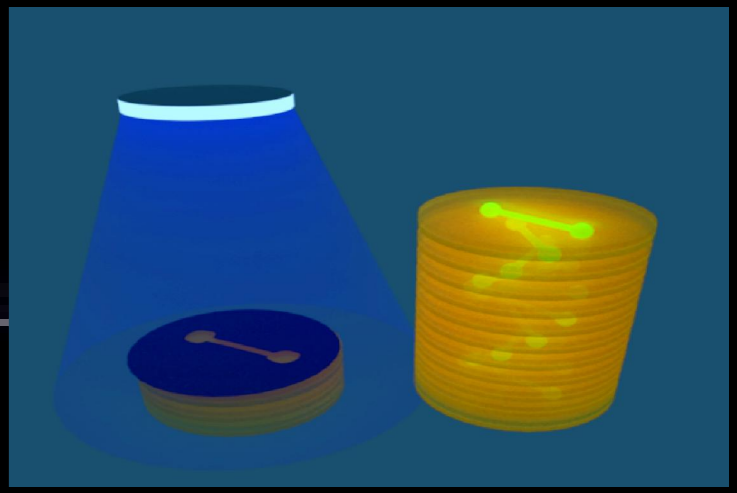


Tissue engineers are trying to "recreate" human tissues & organs that exist.

What is Bioprinting?

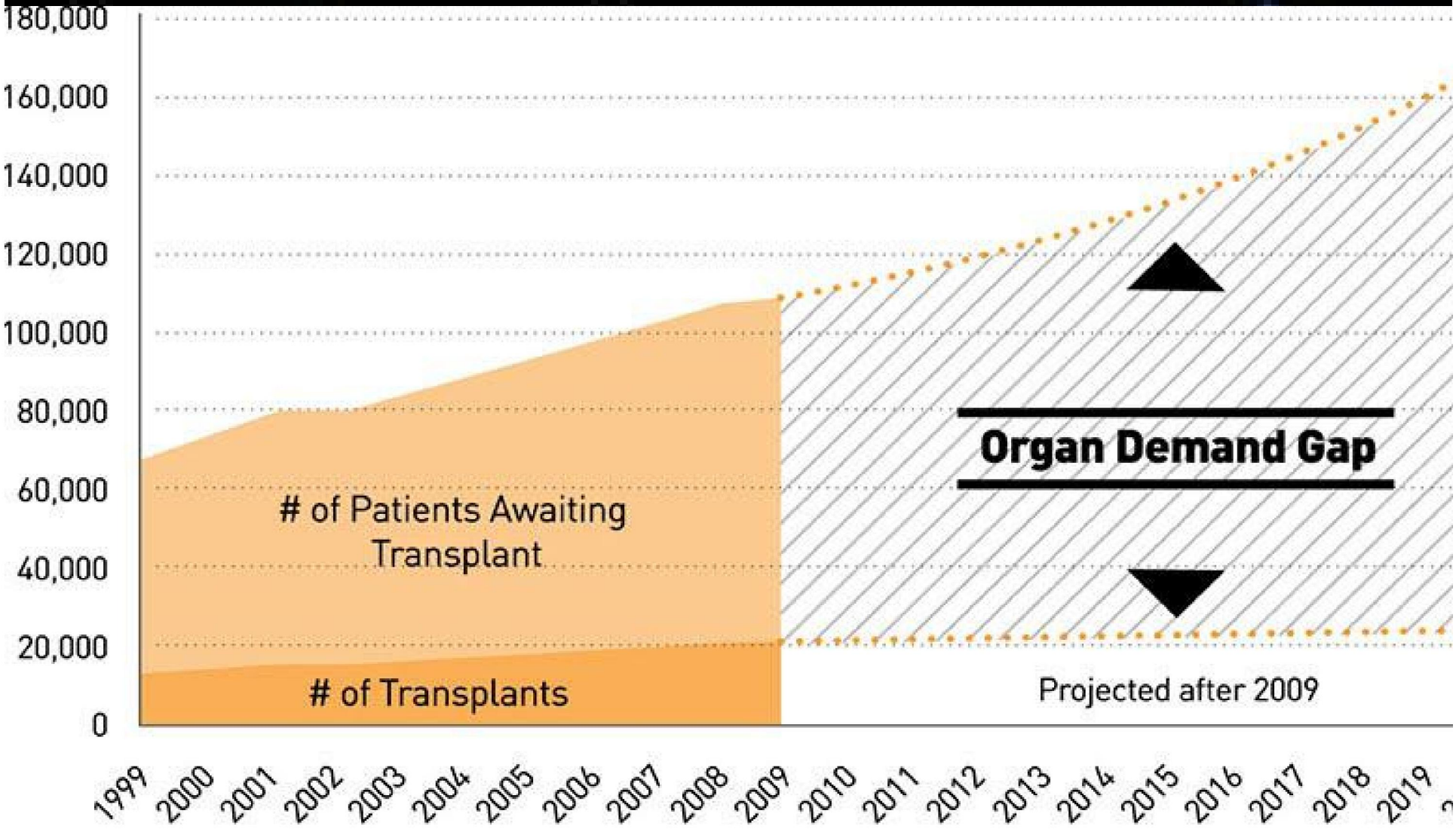
Biomedical application of Rapid Prototyping or additive manufacturing





computer-aided robotic layer by layer additive biofabrication of functional living tissue constructs

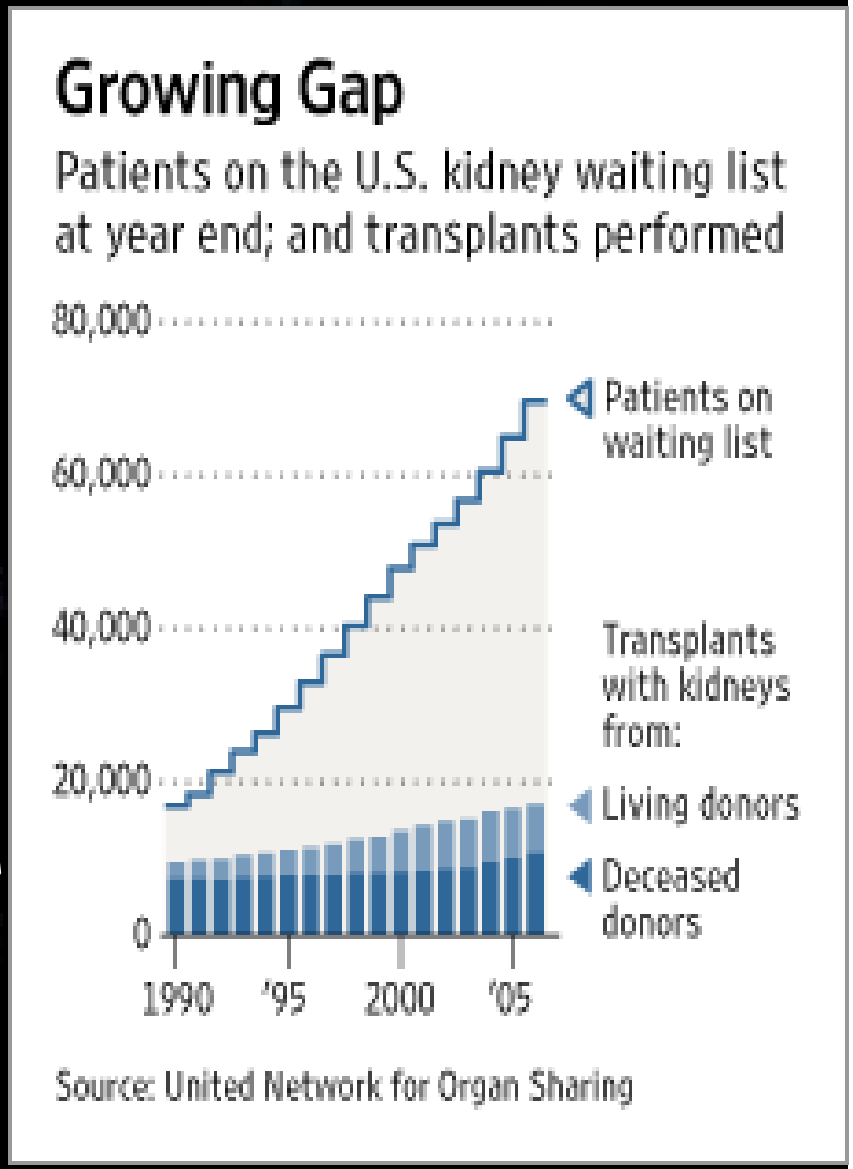
Organ Demand Gap



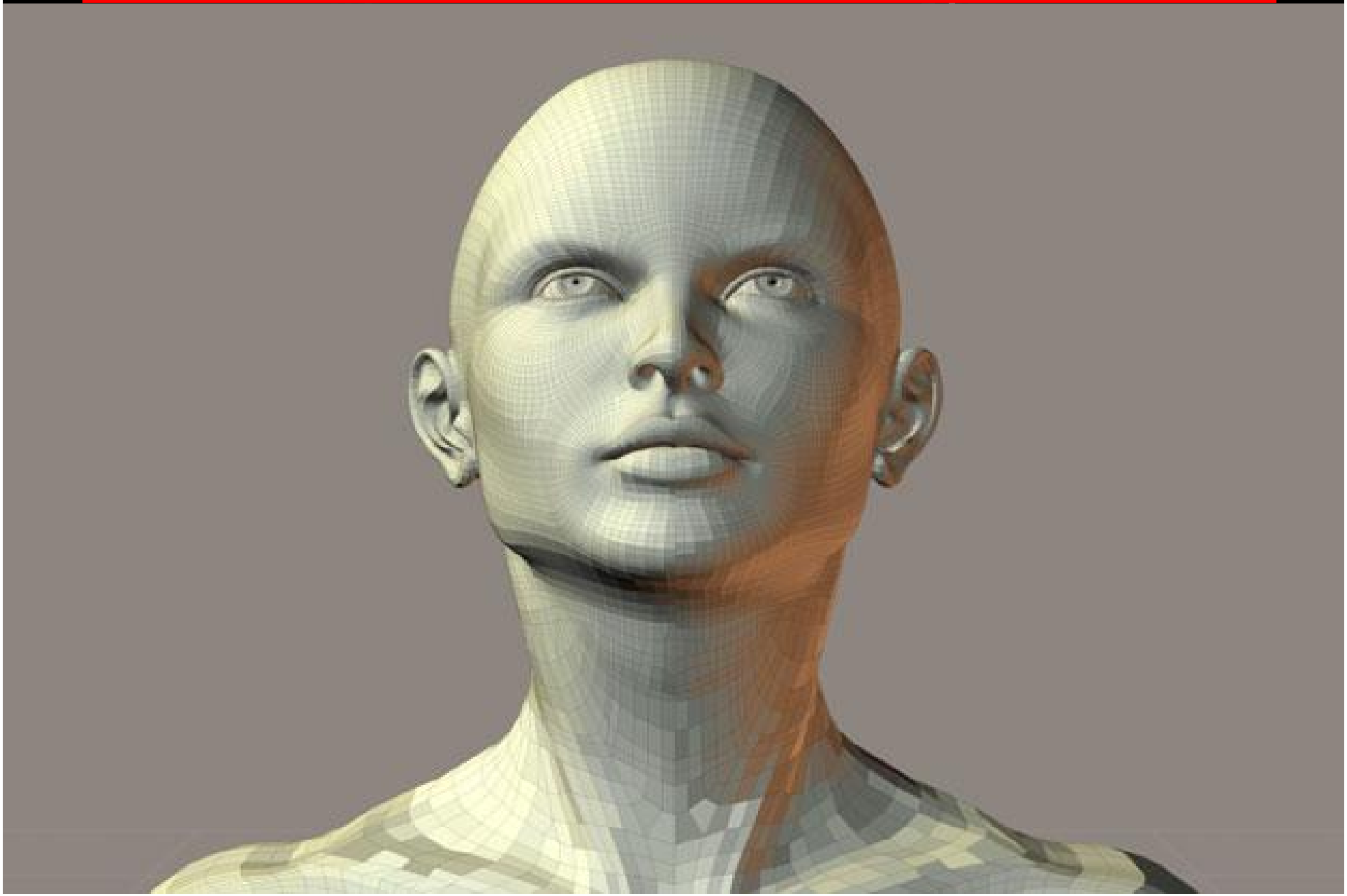
Organ Shortage

Organ shortage has become a demanding problem .
Currently less than a third of US In need of kidney transplant receive it .
There are 100 000 patients in the USA alone who are waiting for kidney Transplantation.

A potential solution to this problem requires manufacturing living Organ from a person's own cells.



Evolution of bioprinting technology

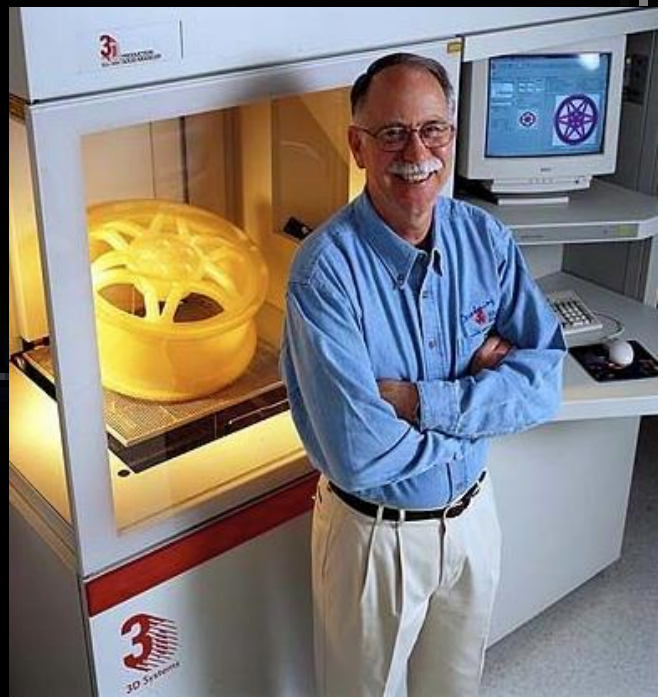


stereolithography

1984: Charles Hull invented stereolithography which made possible creation of a tangible 3D object from digital data.

It is an additive manufacturing technology used for producing prototypes and production parts.

where successive layers of material are laid down in different shapes



Rapid Prototyping (RP)

Now the technology is used in many diverse fields such as jewelry, footwear, industrial design, architecture, engineering and construction (AEC), automotive,aerospace, dental and medical industries,etc.

LayerWise, a metal manufacturing company, created the first-ever 3D printed jawbone, to be installed as the mandible for an 83-year old English woman.

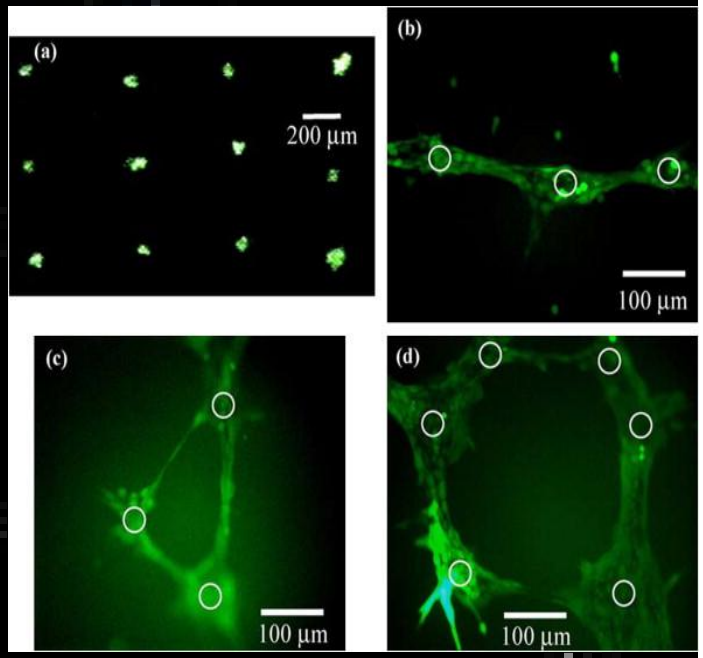
cytoscription

1994:Robert Klebe introduced

Cytoscription:

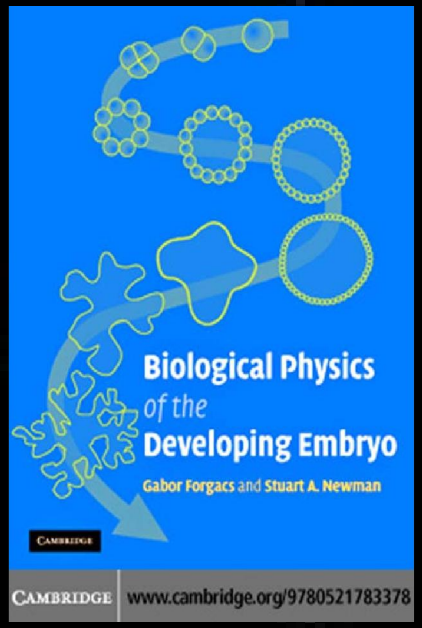
Computer controlled

precise micropositioning of cell adhesion proteins and cells



Principles of tissue self assembly

1996: Gabor Forgacs team observed that cells stick together and move together in clumps with liquid-like properties during embryogenesis.

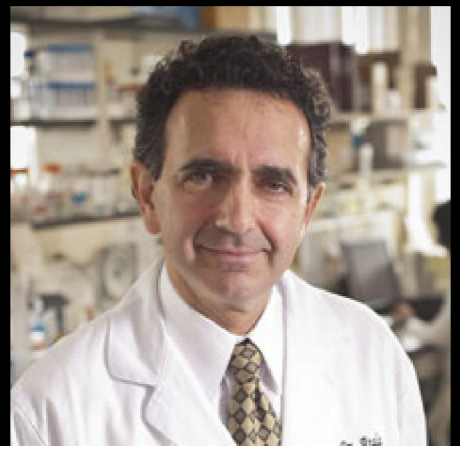


Dawn of Regenerative Medicine

2001: Anthony Atala from Wake Forest university

Treated Lucas Massella a 6 year old child

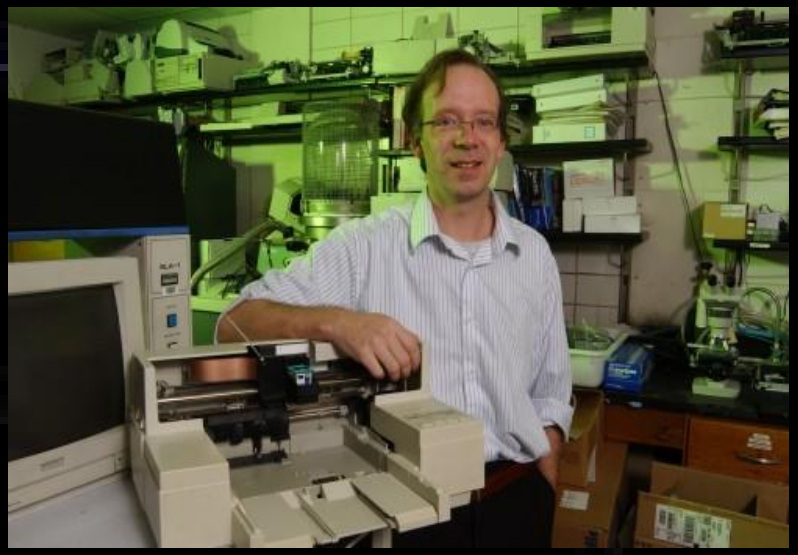
Suffering from spina bifida and complete urinary incontinence with bladder augmentation using a synthetic scaffold seeded with patient own cells (First tissue engineered bladder)



First Bioprinter

2004 : Thomas Boland from Clemson University modified inkjet printer to accommodate and dispense cells in scaffolds.

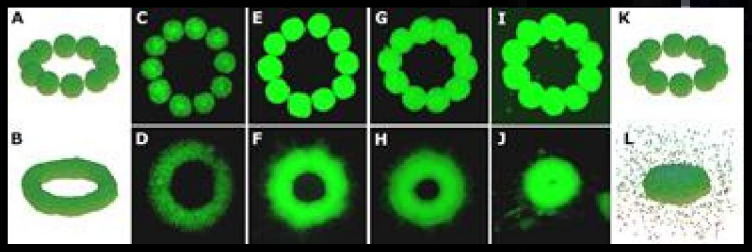
The breakthrough with this technology is that cells now can be precision-placed virtually instantaneously with the materials that make up a scaffold to hold the cells in place.



Scaffold free tissue engineering

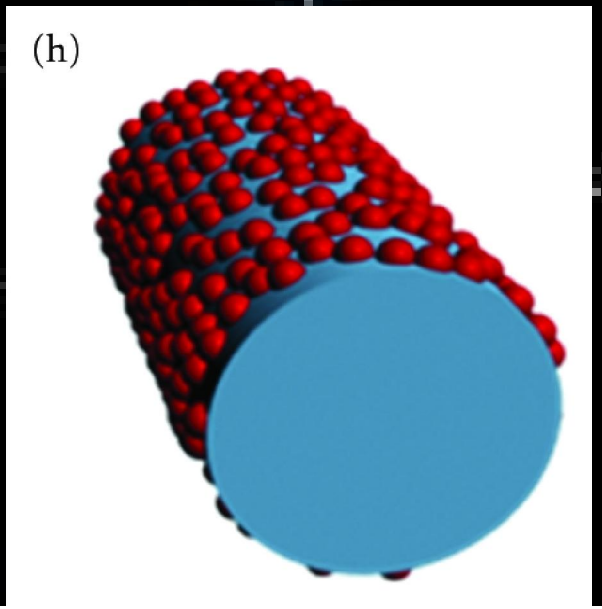
2004 : Gabor Forgacs developed new technology to engineer 3D tissues with only cells ,no scaffold.

http://forgacslab.missouri.edu/bioprinting.html



Tissue Spheroids

2008: the concept of tissue spheroids developed by Mironov et. al as Building blocks in organ printing.



First commercial Bioprinter

2009: First commercial bioprinter (Novogen MMX) created by Organovo Using Forgacs method.

- 2010: First
- Human blood
- Vessel made
- Without using
- scaffold.
- 2011:multiple

drug discovery Platforms and 3D Disease models Developed from Human cells.

Tissue Engineering yields

For the past three decades, tissue engineering has emerged as a multidisciplinary field involving scientists, engineers, and physicians, for creating biological substitutes mimicking native tissue to replace damaged tissues or restore malfunctioning organs .

significant success has been achieved both in research and clinical applications of various tissue engineered grafts like skin, cartilage, bone and bladder.

TE products on market

- A number of products based on tissue-engineering principles are already being used to treat patients, in clinical trials or as FDA-approved therapies.

  Examples:

- SKIN: Epicel, a permanent replacemenfrom the patient's own skin cells and intended to treat burns.
- CARTILAGE:Carticel, is an injectable suspension of cartilage repairing chondrocytes derived from the patient and cultured with growth-promoting factors.

- VESSEL PATCH: Vascugel,currently in clinical trials, is a construct made of donor endothelial cells and designed to be placed on top of an injured blood vessel.

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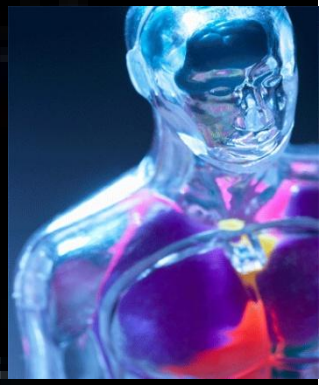
Global market of TE products reached nearly $60 billion in 2010 and will reach $90 billion as of 2016.

Bioprinting Technology



Bioprinting is A computer-aided bioadditive manufacturing process to deposit living cells together with hydrogel-based scaffolds for 3D tissue and organ fabrication.

It offers great precision on spatial placement of the cells themselves, rather than providing scaffold support alone .

Futuristic Organ printing concept

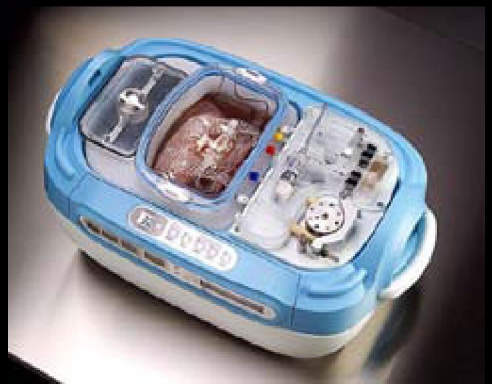
3D functional organs are printed from the bottom up using multiple living cells in supportive media and stored in cartridges .

The cells are printed layer by layer using inkjet printing technology.

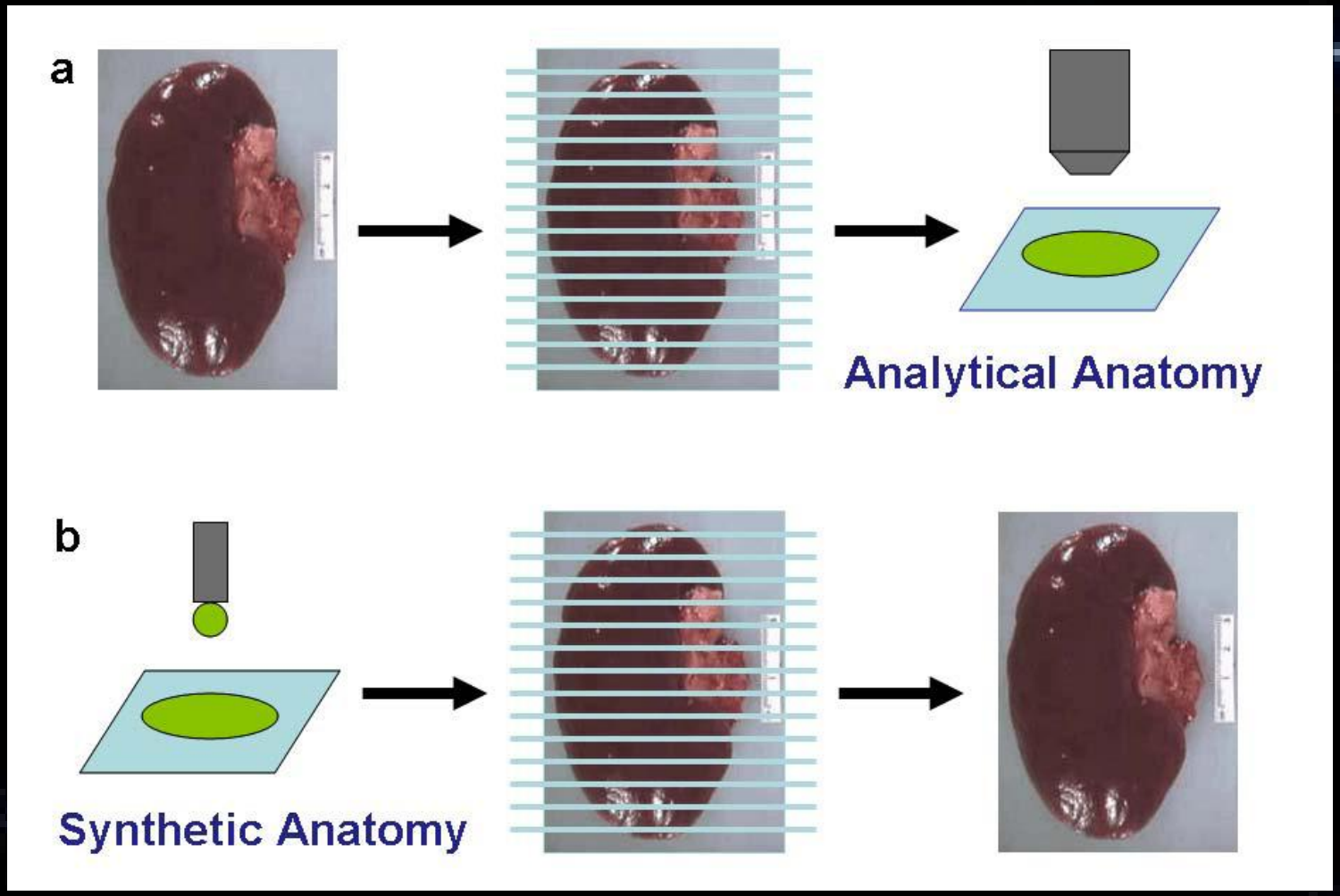
It offers a controllable fabrication process, allowing precise placement of various biomaterial/cell types simultaneously according to the natural constituents

Bioprinting Components

- 1-Pre-processing:
- Bioimaging
- CAD
- Blueprint of tissue
- 2-Processing:
- Bioink +Bioprinter+Biopaper
- 3-Post-processing:
- Bioreactors
- (biomonitoring+tissue
- maturation)



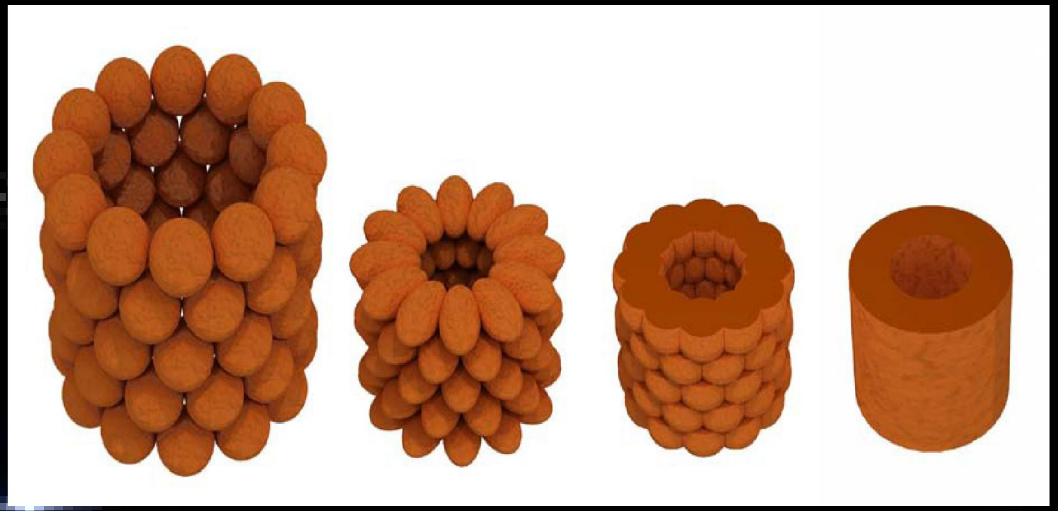
From Analytical to Synthetic Anatomy

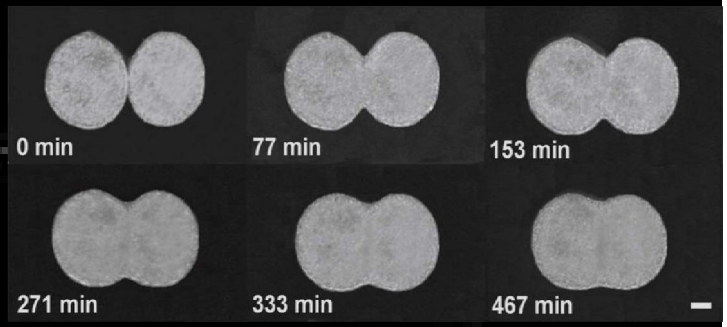


Bioink

- Self –assembling fluidic tissue spheroids made by tissue fusion constitute bioink.

- tissue fusion is a fundamental principle of organ printing technology .





Advantages of tissue spheroids

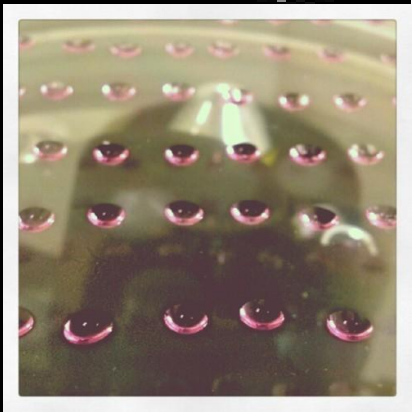
- significantly enhance viability of stem-cell-derived organ-specific cells.

- greatly reduces shear-stress-induced cell damage compared to printing cells directly loaded within biomaterial media

Significance of tissue fusion

- Figure shows hanging drop cultures, where each spheroid contains 20,000 chondrocytes, and fusion between spheroids was observed seven days after harvesting, resulting in a larger-scale cartilage tissue

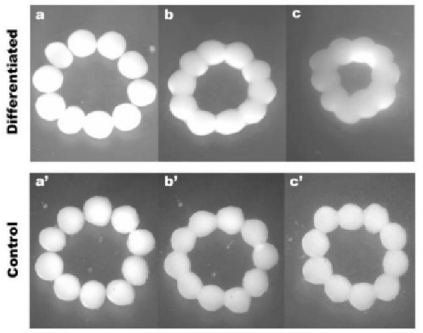
- This shows a great promise in obtaining larger-scale cartilage formation, where researchers have already demonstrated regeneration of cartilage tissue on rabbit knees comparable with natural cartilage tissue.
- Tissue spheroids in that study eliminated or minimized inclusion of biomaterials and hence tissue regeneration achieved without need of scaffolds.
- large scale tissues can be easily obtained by fusion process, while cells in cell/laden hydrogels cannot easily fuse .



Stem cells as a source of bioink

- **Adipose tissue derived** Mesenchymal stem cells are clinically relevant cell source for use as bioink in organ printing.
- 3-dimensional constructs of AD derived MSCs **contract in response to Angiotensin** administration to assemble tissue.

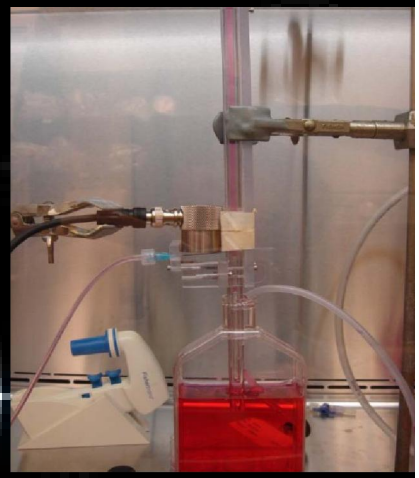
Clinical cell sorters

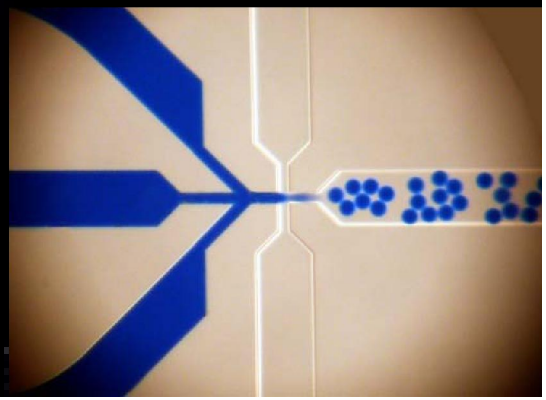


Microfluidics

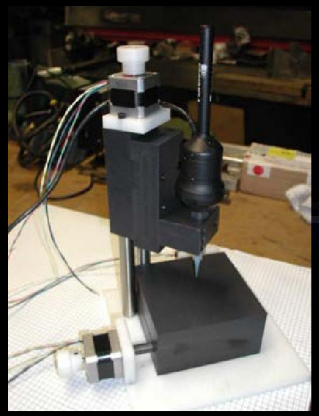
- Digital (Droplets) Microfluidics
- Robotic Acoustic excitation based droplet generation
- Microfluidics is the science of designing, manufacturing and formulating devices and processes that deal with volumes of fluid on the order of nl and pl . The devices themselves have dimensions ranging from mm down to μm.
- Digital microfluidics is an alternative microfluidic technology based on design and manipulation of discrete droplets and bubbles, using principles of emulsion science.

The aim is to create fluid-fluid dispersion into channels (principally water-in-oil emulsion).





Robotic bioprinters



Neatco-1(A), Neatco-2(B), Sciperio/nScript(C)

3D Bioplotter

3D Bioplotter® (designed by envisionTEC GmbH, Gladbeck, Germany

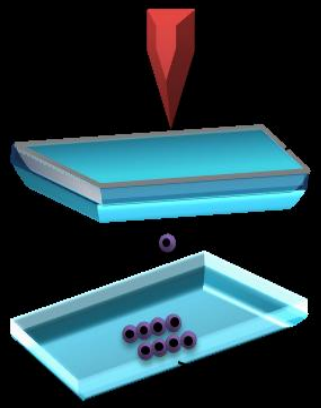
Current Bioprinting Systems

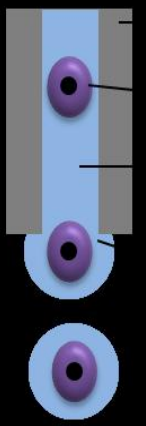
Bioprinting systems can be primarily classified as:

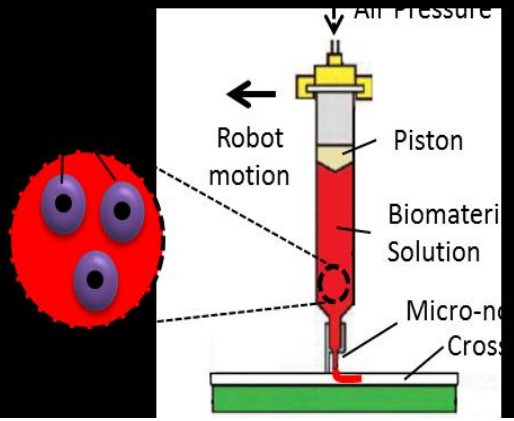
(1) laser-based

(2)inkjet-based

(3)extrusion-based







Laser-based Bioprinters

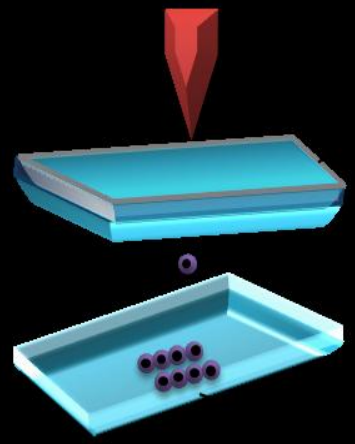
Laser direct-write (LDW) was introduced in 1999 by Odde *et al. to process 2D cell patterning .*

It rapidly creates precise patterns of viable cells on petri dishes.

A laser pulse creates a bubble with shock waves, which propels cells towards the collector substrate

Micro-scale cell patterning is achieved by optimizing **viscosity of bioink, laser printing speed, laser energy, and pulse frequency**

Writing of multiple cell types is feasible by selectively propelling different cells to the collector substrate .

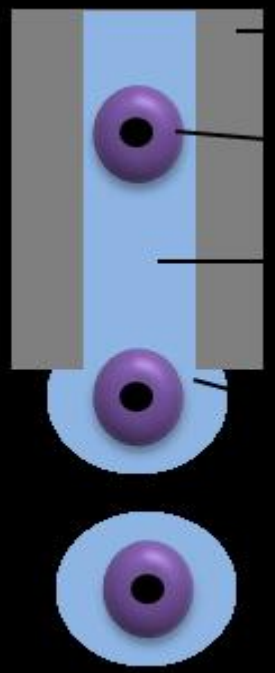


Nahmias *et al.* (2010) successfully performed **Hepato-Cytes patterning** in collagen using LDW .

Ink-Jet based Bioprinters

Introduced in the early 2000s and built a great foundation for future organ printing technologies .

living cells are printed in the form of droplets through cartridges instead of seeding them on scaffolds.



Boland *et al.* **(2006)** *used a thermal inkjet printer* to successfully fabricate 3D cellular assemblies of bovine aortal ECs with thermosensitive gels with high cell viability and maintained cell phenotype .

Ink-jet technique takes digital data from a computer representing tissue or organs, and reproduces it onto a substrate using "bio-ink" made of cells and biomaterials.

Cui et. al **(2012)** applied inkjet printing to repair human articular cartilage, showing its promising potential for high-efficiency direct tissue regeneration .

Inkjet-based system controls parameters such as cell concentration, drop volume, resolution, nozzle diameter and average diameter of printed cells.

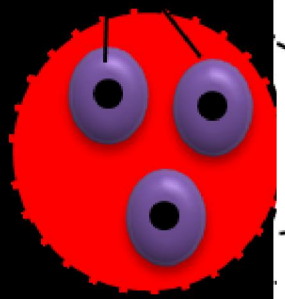
Huang et. al **(2012)** developed a bipolar wave-based drop-on-demand jetting. Cell-encapsulated alginate microspheres were jetted and assembled to create **vertically oriented, short, tubular structures** .

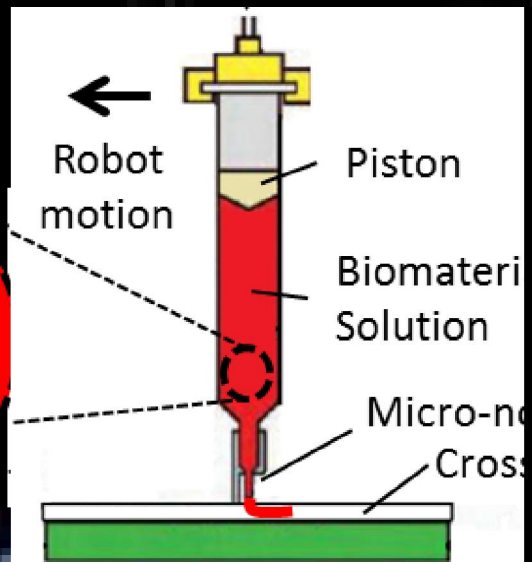
Xu et al. **(2012)** developed a fabrication platform with a different working principle as opposed to traditional inkjet printers, where droplets of crosslinker chemical were deposited onto a suspension of cardiomyocytes and alginate for 3D cardiac pseudo tissue fabrication.

Extrusion-based Bioprinters

- It is based on the extrusion of continuous filaments made of biomaterials. It is an automated three-axis robotic system for extrusion and printing, respectively.
- biomaterial is dispensed by a pressure-assisted system, under the control of "robots," resulting in precise deposition of cells encapsulated in the cylindrical filaments of desired 3D structures

- Wang *et al.* used hepatocytes and
- adipose-derived stromal cells
- (ADSCs) + gelatin/chitosan
- hydrogels to
- engineer an artificia
- liver by (2012) this
- Technique.





Novogen MMX Bioprinter

- Novogen MMX BioprinterTM **(2011)** developed the technology that the printer head has both suction and ejection capability enabling automatic loading of suspension through the nozzle tip occasionally instead of loading it manually prior to fabrication

Novogen MMX BioprinterTM

(designed by Organovo, San Diego, California, USA)

Merits and Shortcomings of various bioprinters

Laser-based systems: high resolution and precise patterning of cells which cannot be achieved by other bioprinting techniques so LDW has a great potential for micro-cellular features in tissues i.e. micro-vasculature.

- prolonged fabrication time, laser shock related thermal / mechanical cell deformation, interactions of cell components with light, gravitational and random setting of cells in the precursor solution, limitations in printing in third dimension and the need for photo-crosslinkable biomaterials should be overcome.
- To improve resolution and precision further, laser pulse features (i.e. pulse duration, wavelength, repetition rate, energy and beam focus diameter), precursor solution properties such as viscosity, thickness and surface tension and substrate properties should be optimized

Merits and Shortcomings of various bioprinters

- To expand this technology in the third dimension, a rotating donor-side carousel leveling system with rotational and linear stages can be developed that allows printing multiple cells in different layers to develop heterocellular structures. Similar technology has been recently demonstrated with multi-material stereolithography.
- Fabrication speed can be improved by increasing the laser pulse rate or integrating multiple laser beams .
- **Ink jet base printers:** Despite their great advantages, inkjet printers suffer from drawbacks including significant cell damage and death as well as cell sedimentation and aggregation due to small orifice diameter that restricts printing cells in high densities ($<5 \times 10^6$ cell/ml) .
- structural integrity of the printed structures is another obstacle, where the droplets do not fuse into each other easily and the shape of droplets cannot be controlled precisely.

Merits and Shortcomings of various bioprinters

- Extrusion-based systems provide better structural integrity due to continuous deposition of cylindrical struts .
- it has several limitations such as shear stress induced cell deformation and limited material selection due to need for rapid encapsulation of cells via gelation. Shear stress on nozzle tip wall induces significant drop in the number of living cells when the cell density is high.This can be partially alleviated using optimum process parameters such as biomaterial concentration, nozzle pressure (ideally minimum), nozzle diameter and loaded cell density.
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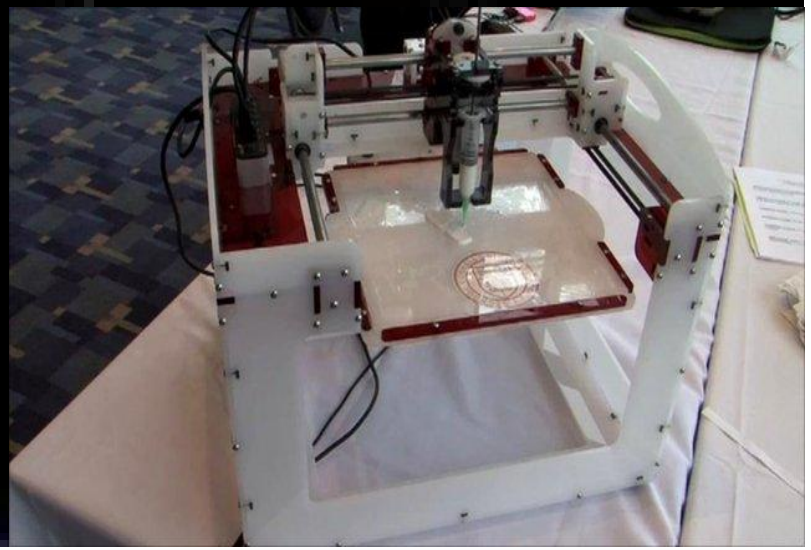
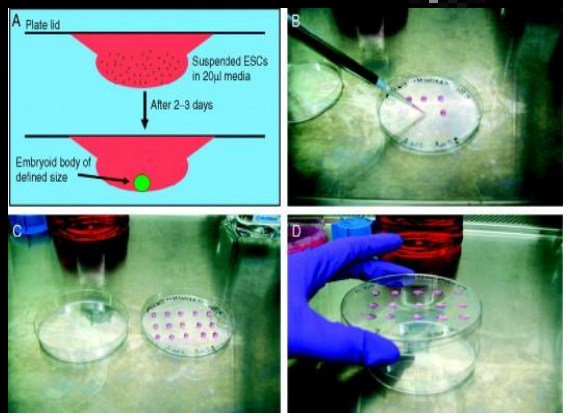


Merits and Shortcomings of various bioprinters

- Restricted biomaterial selection and low resolution and accuracy brings limited applicability of extrusion-based systems . Besides, sufficiently high viscosity is essential for the biomaterial suspension to overcome surface tension-driven droplet formation and be drawn in form of straight filaments. High viscosity, on the other hand, triggers clogging inside the nozzle tip and should be optimized considering the diameter of the nozzle tip.
-
- one of the important disadvantages of encapsulating living cells in biomaterials is that cell-biomaterial suspension needs to be stored considerable time in the material reservoir that compromise cell viability and limits their bioactivity. Thus, a more automated way of loading and ejecting cell-biomaterial suspension is required for scale-up tissue and organ fabrication

Biopaper

- Gelation of cell encapsulated hydrogels is a crosslinking reaction initiated by light, chemical or thermal transitions.
- Photo-crosslinking processes compromise cell viability and pose significant limitations on encapsulation of cells within hydrogels
- Thermal transition crosslinking limits applicability of hydrogels and is not easy to handle after the process while temperature changes can result in rapid degradation of the printed thermogel that does not support cell viability in cell media culture.
- Chemical crosslinking can compromise cell viability if any abrupt pH changes are observed; however, noninvolved crosslinking, i.e. sodium alginate, occurs gently under mild conditions -acid and at room temperature without producing any toxic components, which has a great potential for tissue engineering.



Gelation of cell encapsulated hydrogels

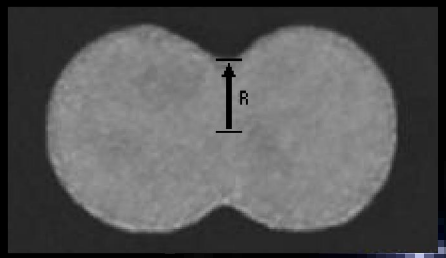
- New hydrogels should be tailored to enhance mechanical properties and processability for specific bioprinting techniques towards advanced tissue and organ fabrication. In addition, these materials should have the ability to withstand sterilization while sterile conditions certainly need to be acquired for process safety

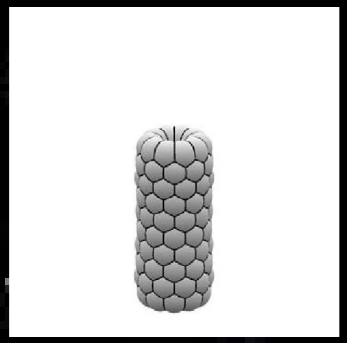
- cell encapsulation in biomaterial allows cell patterning that has a great potential for direct organ printing; however, subsequent extracellular matrix (ECM) formation, digestion, and degradation of biomaterial matrix and proliferation of encapsulated cells are not trivial to control

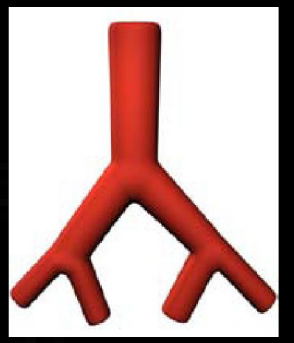
- a new concept was introduced by Mironov et. al **(2008)** with great potential in overcoming issues with cell encapsulation within hydrogels. They proposed tissue spheroids as building blocks for organ printing, where tissue spheroids direct self-assembly toward organ fabrication using hang-drop culture

How to print an intraorgan branched vascular tree ?

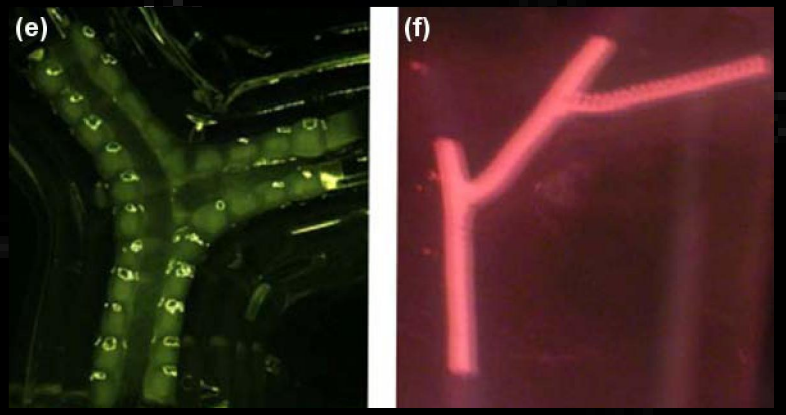
- from self-assembling vascular tissue spheroids







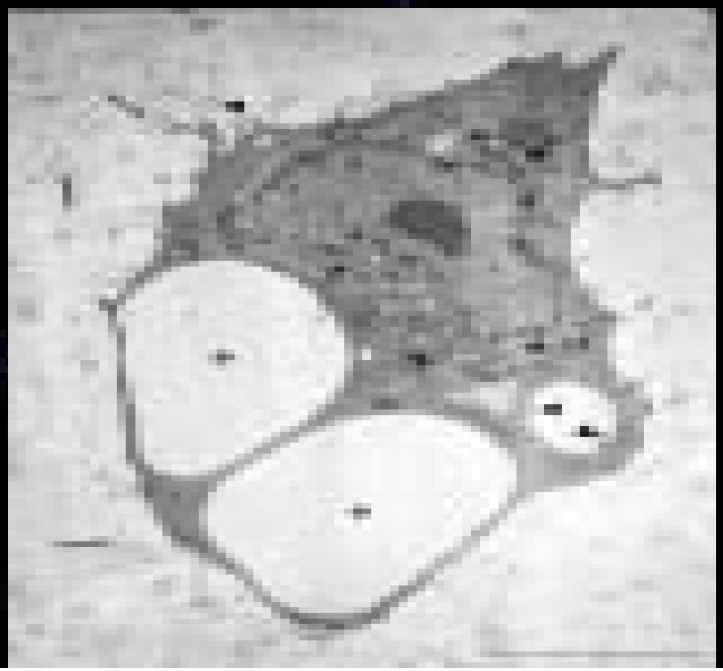
Bioprinting of Vascular Tree

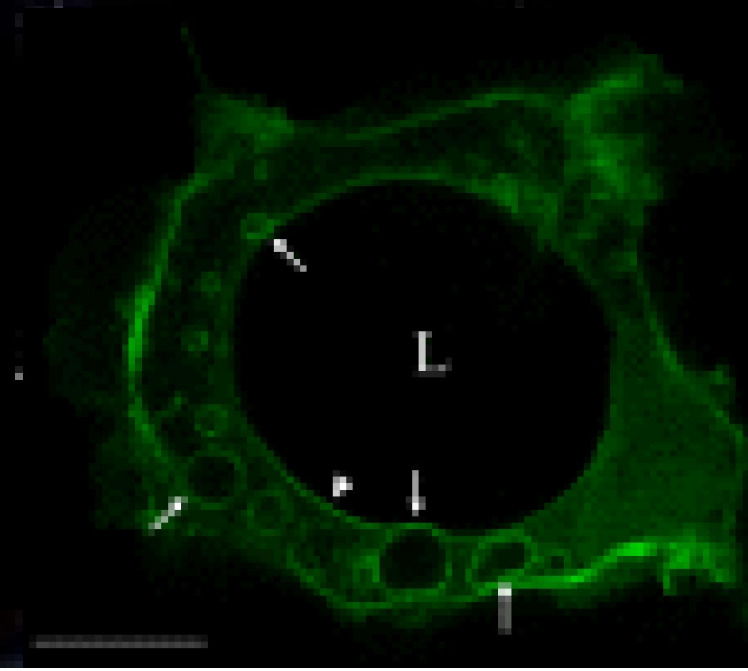


featured in 'Nature News' 2008

Mechanisms of lumen formation

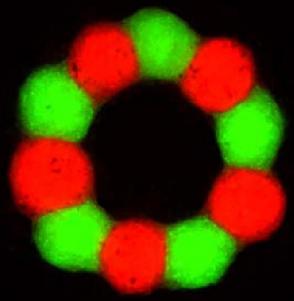
- Endothelial tubes assemble from **intracellular vacuoles** in vivo.
- Kamei M, Saunders WB, Bayless KJ, Dye L, Davis GE, Weinstein BM.
- Nature. 2006 Jul 27;442(7101):453-6

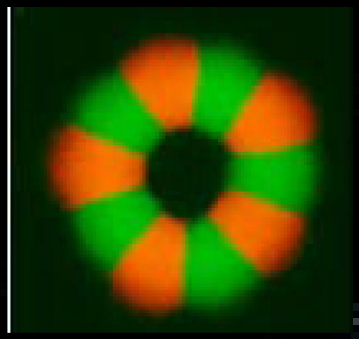




3D bioartificial blood vessels

- Tissue fusion without cell mixing and compaction of vascular tissue spheroids





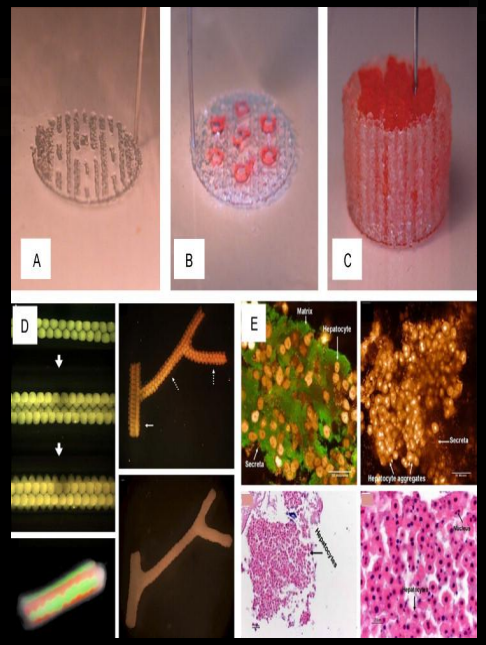
Artificial blood vessels made on a 3D printer may soon be used for transplants of lab-created organs

Tovar et. al (2011) Fraunhofer Institute, Germany

Blood Vessel printing

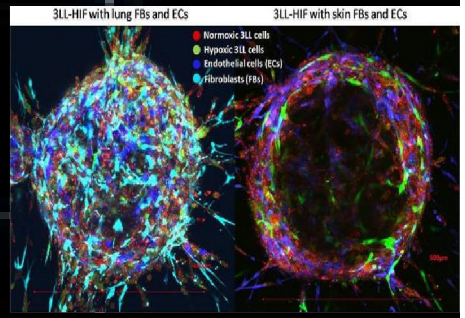
- By using tissue spheroids, Forgacs et al. **(2009)** at University of Missouri, Columbia, used RP-based bioprinting together with multicellular spheroids made from smooth muscle cells and fibroblasts, producing scaffold-free vascular constructs

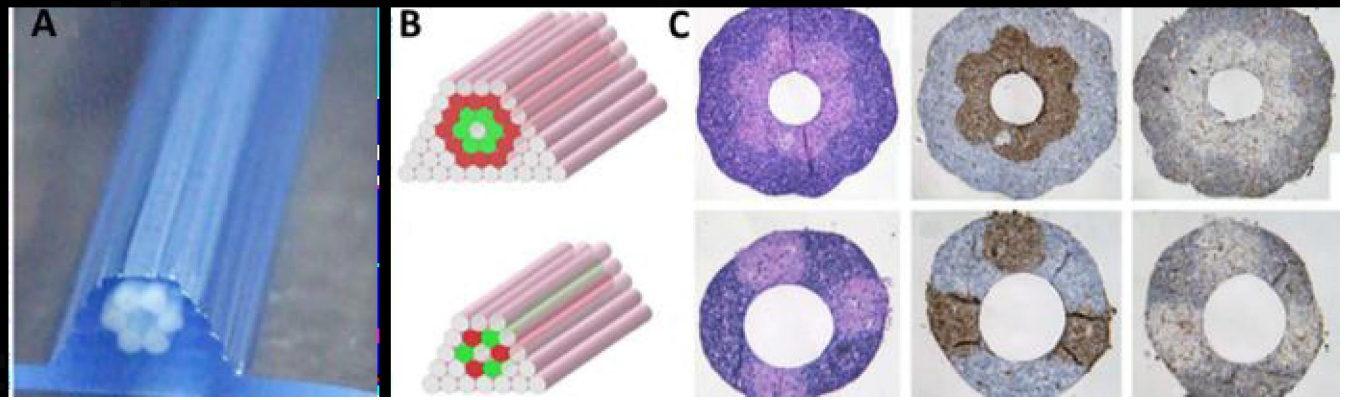
- Figure illustrates vascular construct printing, where tissue spheroids are printed sequentially in cylindrical filaments from the bottom up. Upon fusion of tissue spheroids followed by a tissue maturation process of three days post-printing, the support material is pulled away manually to generate the lumen. Multiple cell types, including human umbilical vein smooth muscle cells and human skin fibroblast cells, were printed together to fabricate multicellular constructs.



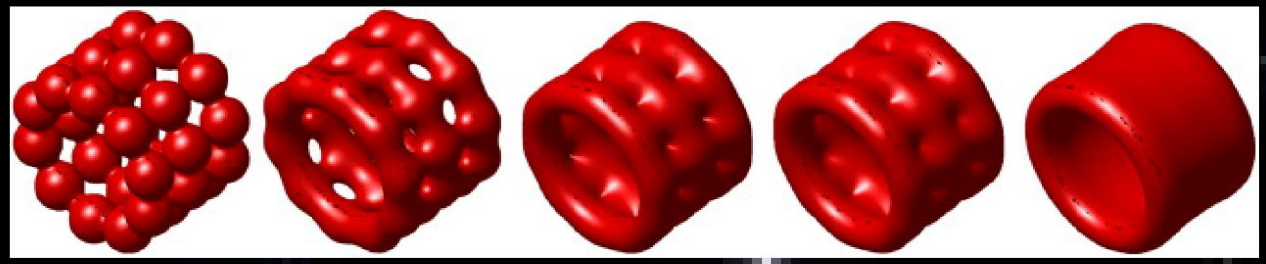
Tissue spheroids for vessel printing

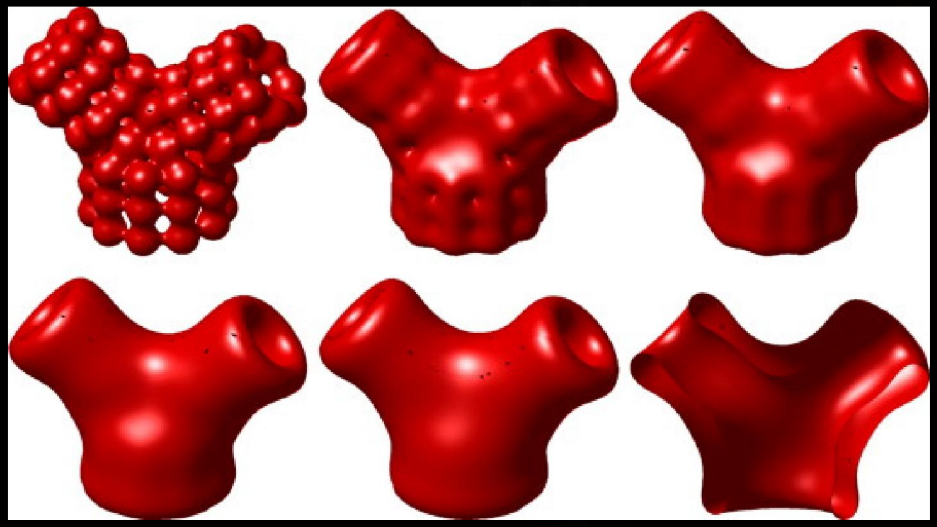
(a) deposition of straight filaments containing a string of tissue spheroids (stained in white) with agarose filaments as support material (stained in blue) both around cellular filaments and inside the core, (b) design for multicellular assembly (c) printed samples with human umbilical vein smooth muscle cells and human skin fibroblast cells





Schematic Blood Vessel Printing Process





Organ Printing

- Organ printing is a computer-aided process that offers a pathway for scalable, reproducible mass production of engineered living organs where multiple cell types can be positioned precisely to mimic their natural counterparts.

It should be ensured that the printed organ functions correctly.

Developing a functional organ

- For this we need advances in and integration of three types of technology
- **(1) cell technology:** which addresses the procurement of functional cells at the level needed for clinical applications
- Success in fabrication of functional organs highly depends on advancements in stem cell technology.

- **(2) biomanufacturing technology:** which involves combining the cells with biomaterials in a functional 3D configuration

- **(3) technologies for *in vivo* integration :** which addresses the issue of biomanufactured construct immune acceptance, in vivo safety and efficacy, and monitoring of construct integrity and function post-implantation.

integration of vascular network

- The most critical challenge in organ printing is the integration of vascular network .

- Without vascularization, engineered 3D thick tissue or organs cannot get enough nutrients, gas exchange, and waste removal, all of which are needed for maturation during perfusion

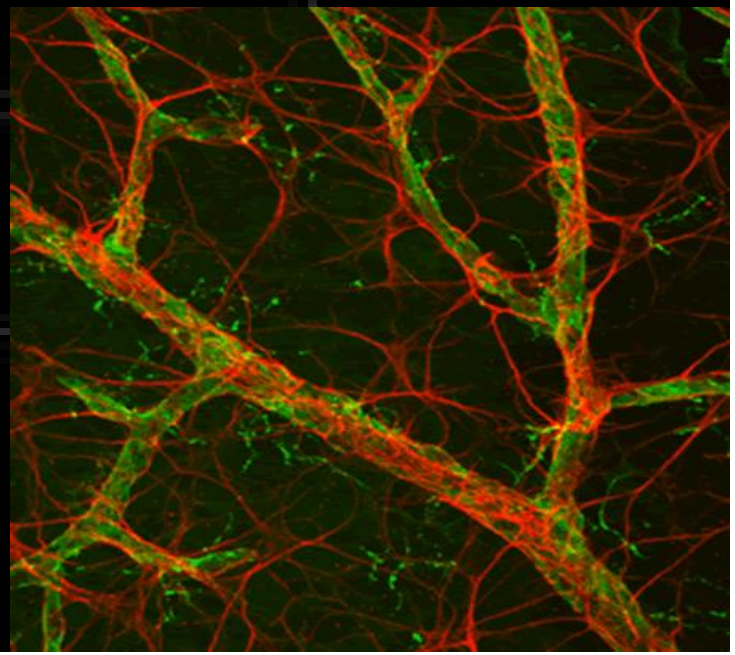
Fabricating bifurcated channels

- Although several researchers have investigated developing vascular trees using computer models, only a few attempts have been made toward fabricating **bifurcated** or branched channels .
- In order to closely mimic natural organs, 3D vascularized organs need to be fabricated using heterocellular aggregates

semipermeable microfluidic channels

- we alternatively propose printable semipermeable microfluidic channels to mimic a vascular network in perfusing media and facilitating oxygenation for cell viability, coaxing tissue maturation and formation

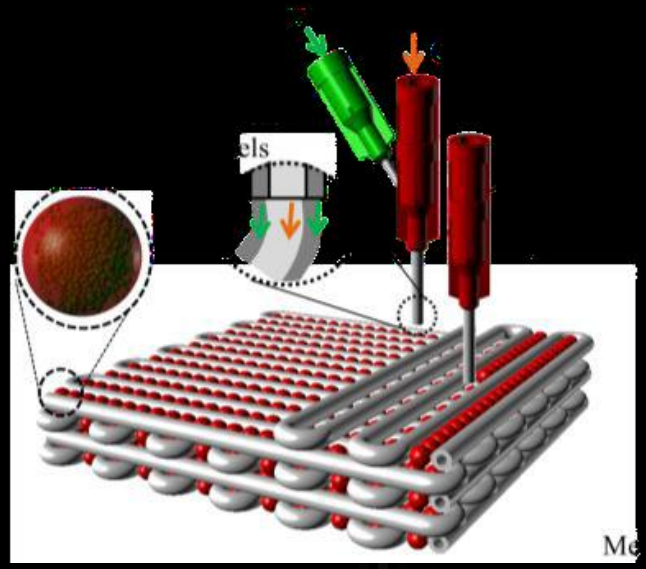
the laser confocal image showing single lumen channel with live/dead staining where CPCs are labeled with calcein AM and ethidium homodimer

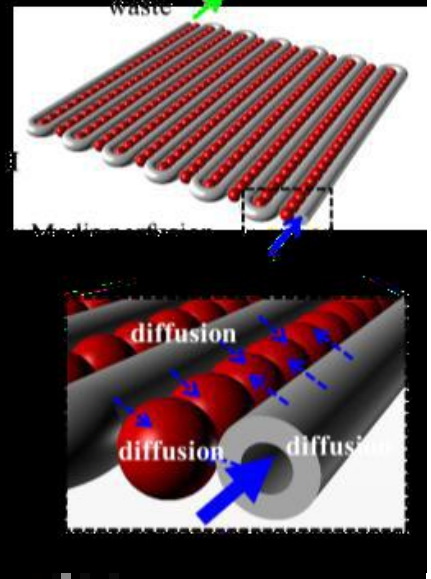


Integrated tissue fabrication with vascular channels

conceptual model of 3D organ printing through integration of vessel-like micro-fluidic channels with cellular assembly .

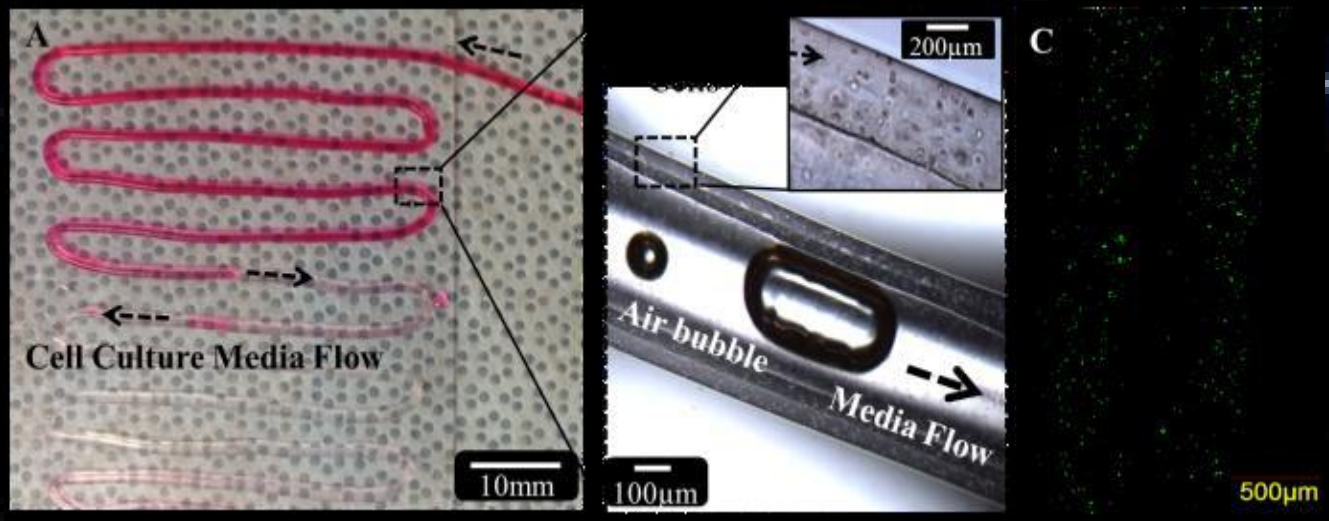
vessel-like microfluidic channels printed in tandem with tissue spheroids layer by layer. The printed structure can be then connected to a bioreactor for media perfusion.





This concept also allows inclusion of multiple cell types in a spatially organized way by integrating another printer unit mounted on the robotic arm to print a secondary type of spheroids precisely.

Printed microfluidic channels



Printed cellular microfluidic channels: (A) perfusion of oxygenized cell type media, (B) media flow with intentionally generated air bubbles and

vessel-like micro-fluidic channels are printed in a 0°-90° lay-down pattern to develop 3D structures.
Oxygenized perfusion media can be pumped into channels for circulation purposes.

After parallel printing of each micro-fluidic channel layer, cellular spheroids, i.e., tissue spheroids or cell encapsulated microspheres, can be deposited between fluidic channels in the form of droplets using another robotic arm;
semipermeable fluidic channels allow transport and diffusion of media to the cellular environment.

Mechanical properties of printed channels

- mechanobiological properties of printed channels are under investigation, and electrospun nanofiber reinforcement is performed to match the mechanical properties with that of blood vessels. Elasticity and tensile strength are essential to be biomimetically mediated
- Viability study of printed tissue is high.
- Advancements in bioprinting heterocellular architectures with precise and selective cell deposition are promising

- In order to fabricate scalable organs such as a mouse liver which has $1.3x10^8$ cells per gram , the bioprinter needs to run for several hours considering the resolution of the system as in microscale.
- sterilization is also crucial during bioprinting

- commercial bioprinters can cost substantially high around $100-200k depending on their unique capabilities, where home-made bioprinters on the other hand roughly cost less than quarter of their commercia l counterparts.

- Another challenge for organ printing technology is the rapid or accelerated tissue maturation process, where printed organ constructs should be rapidly fused, remodeled, and maturated toward a solid construct, ensuring mechanical rigidity for transplantation.
- production and deposition of collagen and elastin during tissue maturation are essential to enhance the mechanical properties of the printed organs

- Then the fabricated structure needs to be transferred to the bioreactor .

Bioreactors

A.

B.

C.

Perfusion Bioreactor for Bioprinted Organs

Automated Bioprinting

- Bioreactors such as an irrigation dripping perfusion bioreactor can be used to expedite tissue maturation and organ formation , providing an optimal environment.

Considering the pathway from isolation of stem cells to transplantation into a human, seamlessly automated protocols and systems are essential for customized functional organ fabrication.

Monitoring the Bioprint product

- imaging modalities such as CT, PET , and NMR imaging should be used to monitor the transplanted organ noninvasively.
- NMR offers a unique advantage in monitoring organ integrity and function without the need to modify the cells genetically

Pathway of organ printing

- (i) blueprint modeling of an organ with its vascular architecture
- (ii) generation of a process plan for bioprinting
- (iii) isolation of stem cells
- (iv) differentiation of stem cells into organ-specific cells
- (v) preparation and loading of organ-specific cells and blood vessel cells as well as support medium
- (vi) bioprinting process followed by organogenesis in a bioreactor for transplantation.

Miniature organs

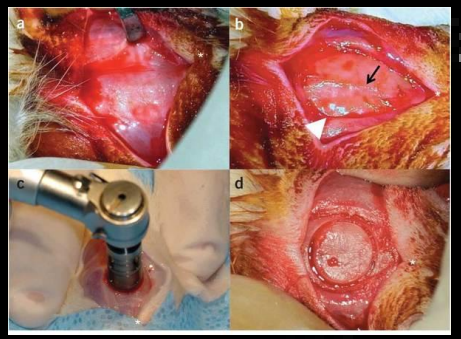
- Miniature organs can be built in smaller scale than their natural counterparts.
- They are a future trend in organ printing and a transition towards fully functioning organs.
- As "a factory in the human body" they perform the most vital function of the associated organ, such as insulin secreted by a pancreatic organ in substantial amounts to regulate the glucose level.
- Insulin secreting ß-cells make only about 2% of pancreas cells .

Miniature organs can also be designed to bring new functionalities and superiorities in the human body, such as living organs that can continuously generate electricity to eliminate the use of batteries for internal devices such as peace makers.

In-situ bioprinting

- Weiss et. al **(2012)** made a multi-head inkjet-based bioprinting platform to fabricate heterogeneous structures with a bottom up concentration gradient . Multiple growth factors were printed with spatial precision in a functionally graded manner into rat calvarial defect *in-situ* .

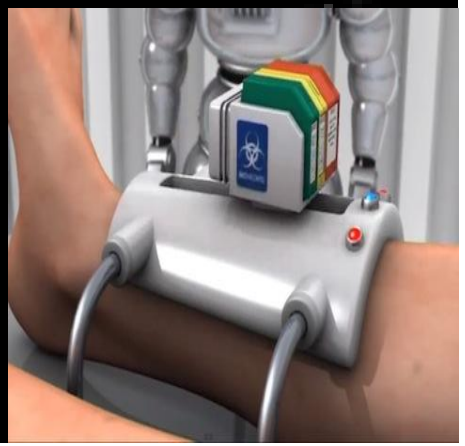
They demonstrated feasibility of **in-situ printing** .



NATURE PROTOCOLS (2012)

In situ printing,

- where living organs can be printed in the human body during surgery.
- Currently, *in-situ bioprinting* has already been tested for repairing external organs like skin .
- With the recent advancements in robot-assisted surgery, computer-controlled robotic bioprinters will lead the evolution of this technology in the very near future.



in vivo bioprinting

- Future of bioprinting technology is bioprinting in situ

[in vivo bioprinting]

RP-based bioprinting :Summary

- Organ printing, is defined as computer-aided additive biofabrication of 3D cellular tissue constructs
- takes advantage of RP technology to print cells, biomaterials, and cell-laden biomaterials individually or in tandem, layer by layer, to directly create 3D tissue-like structures.

- Tissue engineering has been a promising field of research, offering hope for bridging the gap between organ shortage and transplantation needs.

- building three-dimensional (3D) **vascularized organs** remains the main technological barrier to be overcome

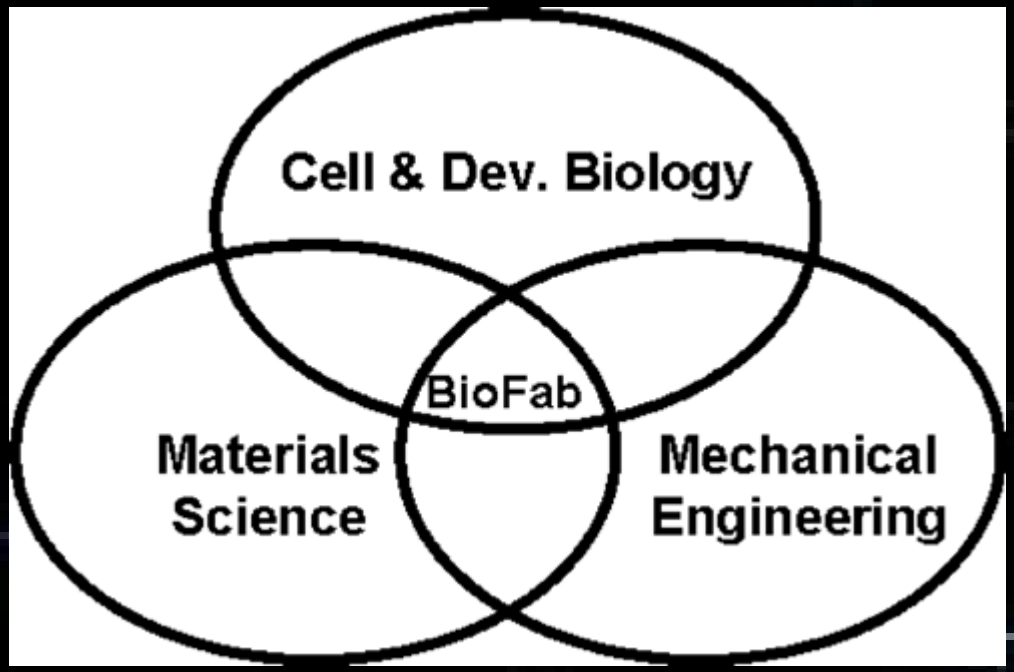
Kidney biofabrication project

$ 20 million NSF grant [2008]

biofabrication

- biofabrication deals with science, engineering and technology or production, based on using living matter as raw material.
- biofabrication is also narrowly defined as the production of complex biological products using living cells, molecules, extracellular matrices, and engineered biomaterials.

A multidisciplinary Sci/Tech



The main disciplines contributing to the emergence of biofabrication: 1-cell and developmental biology 2- mechanical engineering 3- biomaterials science.

Role of mathematical modeling / computer simulation

- Modern fabrication is unthinkable without employing sophisticated mathematical modeling and computer Simulation.
- mathematicians,computer engineers, and mechanical engineers use their mathematical modeling and computer simulation skills in biofabrication.
- Biofabrication from the start must be a predictable technology and built on predictable models and measurable parameters .

Both the designing process and the assembly process, including designing the assembly line and final product testing, is performed *in silico.*

- For BLUEPRINT creation: open access CAD software packages posted on the fab@home website.
- To predict permeability and the mechanical properties of fabricated scaffolds and tissue constructs: finite element analysis software packages
- Examples:
- the extrusion process can be effectively modeled with MoldFlow software
- Tissue assembly can be modeled with the molecular dynamics and Surface Evolver software
- Perfusion in bioprinted constructs created from partially fused tissue spheroids can be modeled and simulated using the lattice Boltzman approach (LBflow software) and computational fluid dynamics software such as Fluent

Animal-free meat biofabrication , Animal-free leather and fur production

- In the long term, tissue-engineered food is the inescapable future of humanity.
- the first international symposium on tissue engineered
- food in Norway in 2008 (http://invitromeat.org/
- content/view/14/29/)
- The world trade in leather, which is one of the most
- widely traded commodities, stands at over US$60 billion a year, and is growing.
- patents issued on in vitro synthesis of collagen by cultured fibroblasts are growing in number.
- Tissue engineered skin for medical applications and drug toxicity is already a clinically proven reality.

Industrial biofabrication of TE food

1—myoblasts—skeletal muscle tissue progenitor cells,

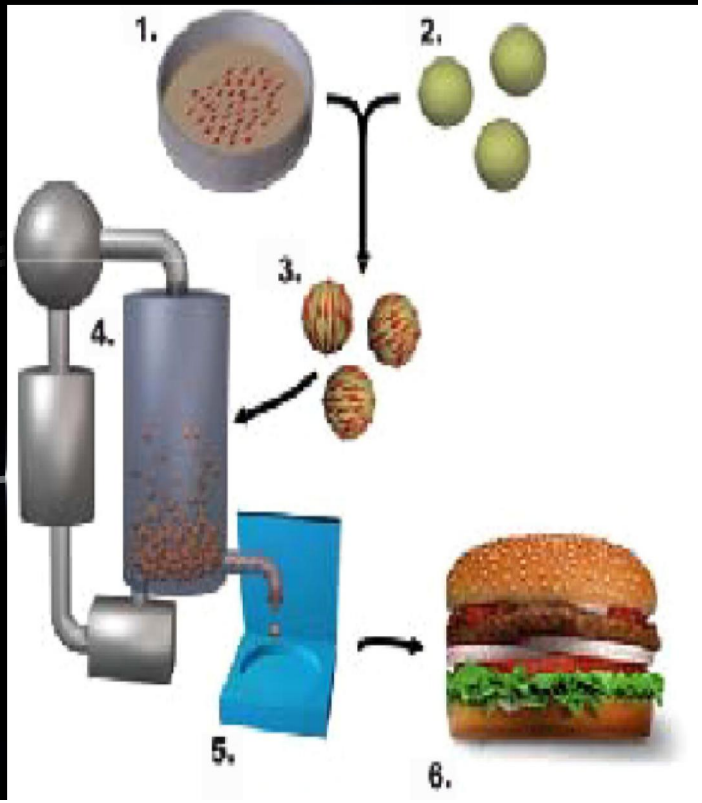
2—porous microspheres from edible polymers,

3—myoblasts seeding on porous microspheres,

4—bioreactor,

5—microwave and

6—hamburger



Biofabrication of skin,leather and fur

- Biofabrication can open up new opportunities for developing large-scale animal-free technology for natural leather and fur production
- The world fur market is around $12 billion and is fast growing due to increase in disposable income in China and Russia.

- The epidermis stem cells have been isolated and growth of hair *in vitro* in cell culture has been demonstrated

Industrial Biofabrication human tissues /organs

- Recently published medical economic data have strongly suggested that a tissue-engineered vascular graft could be sold for $25–30K and may even under certain conditions sell for $50K .
- A $250K price for a tissue engineered kidney is economically justifiable
- Kidney biofabrication alone can potentially create a $25 billion market .
- Biofabrication of living organs can save thousands of human lives and dramatically reduce the cost of health care.
- investment in artificial human organ biofabrication technologies is both economically and socially justifiable and morally sound.

Biofabrication of extracorporeal living tissue including devices

- Scalable production of living tissue spheroids from isolated human cells and their integration into extracorporeal devices is a biofabrication challenge
- extracorporeal liver and kidney devices for the treatment of some acute diseases and toxicological conditions could be life-saving procedures.
- Most advanced extracorporeal tissue-based devices are extracorporeal liver and extracorporeal kidney devices

In vitro 3D tissue models of human diseases

- Biofabricated 3D *in vitro models of human diseases could be* superior compared with traditional 2D *in vitro cell culture on* Petri dishes as well as with animal *in vivo studies.*

Drug toxicity and drug discovery assays

- Tissue-based drug discovery assays must help to identify and validate potential therapeutic targets, Tissue-based drug toxicity assays are dealing primarily with toxicological aspects of already selected therapeutic targets or compounds.
- The superiority and higher level of authenticity of 3D tissue assays as compared with 2D assays are already proven.
- robotically biofabricated will become a powerfull high content and high throughput tool for drug discovery and drug toxicity studies.

Biosensors and bioreports in space research

- Even in the case of planned Moon and Mars manned missions, the development of sophisticated cell and tissue-based bioreporters and biosensors is essential to check radiation safety.
- Sophisticated miniaturized tissue-based radiation biosensors and microfluidic bioreporters have already been
- developed for NASA.

Bioart

- Rapid prototyping is already broadly used in art and jewelry and it is logical to assume that biofabrication technology will also bea pplied in bioart.
- Growing semi-living tissue-engineered sculptures has already been employed in an art project by provocative bioartist Oron Catts (http://www.symbiotica.uwa.edu.au/) who considers tissue engineering as a new art medium .

Biogames

- An attempt to create a self-assembled moving microdevice driven by tissue engineered muscle has already been reported
- using biofabricated living devices for personal entertainment is also technologically feasible , but this is an extremely controversial and culturally/morally sensitive issue

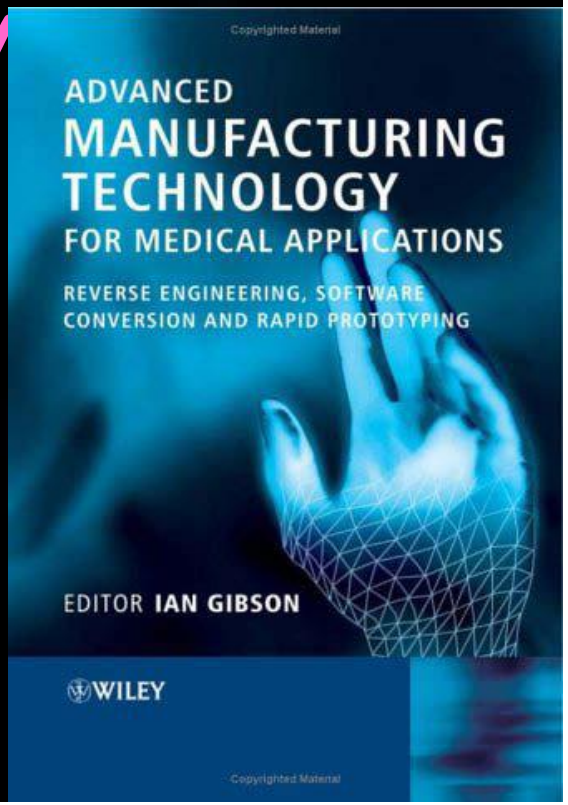
Strategies to advance biooprinting science and technology

1- We need training course in Biomedical Applications of Rapid Prototyping

2-We need to build necessary infrastructure for emerging field of Bioprinting/Biofabrication

3-We need to establish a research society on

- "Biofabrication"



Strategies to advance biooprinting science and technology

4- We need conferences on "Virtual Tissues" and "Digital Human"

5-We need Development of new curriculum
and new Bachelor Program in **Biomedical Rapid Prototyping**

6-We need universities to train multidisciplinary "physician scientists" not Only clinicians.

Essential steps in building a biofabrication research community

- The organization of a global biofabrication research community is the first and probably the most essential step in ensuring the emergence of future biofabrication technology and industry.
- Such a process usually takes several years and is driven by recruitment to emerging a critical mass of experts and scientists from different disciplines

- The indicators of the evolution of a new research community around a new research field include :

  1-the emergence of research groups and specialized research centers;

  2-organization of special sessions, symposiums, conferences,and congresses + publication of new journals and textbooks;

  3-development of training courses;

  and, finally,

  4-organization of professional societies.

Toward biofabrication industry

- The lead time for knowledge to become applicable technology and begin to be accepted on the market is between twenty-five and thirty-five years'.
- knowledge-based innovation is never based on one factor but on the convergence of several different kinds of knowledge
- Knowledge-based innovation requires careful analysis of all the necessary factors, the knowledge itself and social, economic or perceptual factors'. Putting biofabrication technology in a broader social and economic context is essential for perceptivity
- into it.
- To be successful, a knowledge-based Innovation there has to be receptivity to it.
- Negative opinion leaders can destroy perception of the biofabrication field and delay its development whereas enthusiastic support from influential leaders and experts and media can dramatically enhance the rapid development of biofabrication

Research Committee on Bioprinting

- because the inherent risk of knowledge-based innovation is so high, entrepreneurial management is both particularly necessary and particularly effective'.
- Any state or country which really wants to be a world leader in 21st century manufacturing must create a network of well-funded and, most importantly, well managed national biofabrication engineering research and technology centers and/or national biofabrication technology development programs.

- Some influential economists predicted that health care will continue to grow, up to 20–30% of USA economy, in the 21st century. So biofabrication could be the dominant paradigm for 21st century manufacturing.

There is no such a thing as science fiction.

There is only science eventuality

Prof. Krummel

Chair of Department of Surgery

Stanford University

The best way to predict the future is to invent it.

Alan C. Kay
fellow at Apple Computer Inc.,