Organism Manufacturing Technogy in China

Dr.-Ing. Peifan Ding

Senior Consultant, CMES

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outline

- Biomanufacturing and organism manufacturing
- Tissue engineering, the rich fruits and the dilemmas
- Direct cell controlled assembly
- Conclusion

BM——Bio-Manufacturing

BM

- <u>Bionic manufacturing</u>—— The science of simulating of the structure and behavior of organism (仿生制造)
- <u>Organic (-mass or-matter) Manufacturing</u> —— The science of manufacturing based on bio-process
(生物质制造)
- <u>Organism Manufacturing</u>—— The science of rehabilitating and manufacturing of organism
(生物体制造)

OM refers in particular to manufacture the organism, namely the tissue and organ, especially the complex organ.

—— Organism Manufacturing（OM）

Definition of OM

Based on the principle and method of modern manufacturing science and life science, diverse cells and cell aggregates are assembled directly and indirectly to manufacture living structures with metabolic functions, after culturing and training, the replacement is formed to repair or regenerate tissue or organ.

The main research direction of OM

- （1）modeling and designing of organism

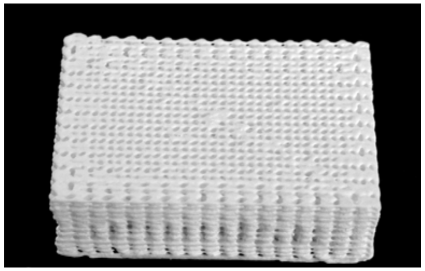
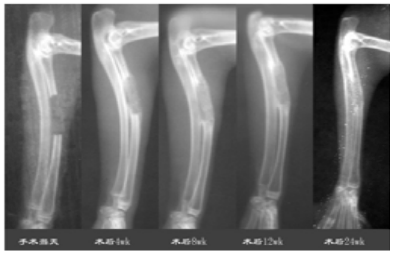
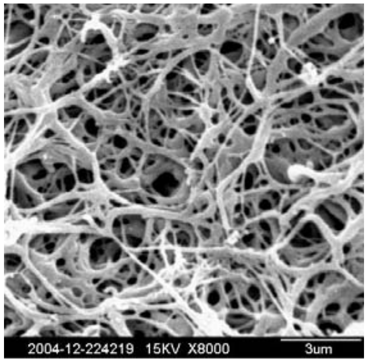
- （2）indirect cell assembly of organism
  ——Tissue engineering

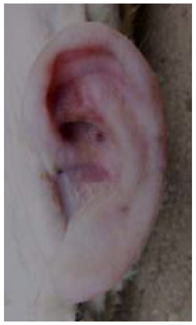
- （3）direct cell assembly of organism

Some examples of Tissue Engineering

- Currently, the research of TE achieves great progress in engineered skin, cartilage, bone, blood vessel, neuron and so on.
- Rich fruits of TE scaffolds of vascular and bone restoration were achieved in Tsinghua University.

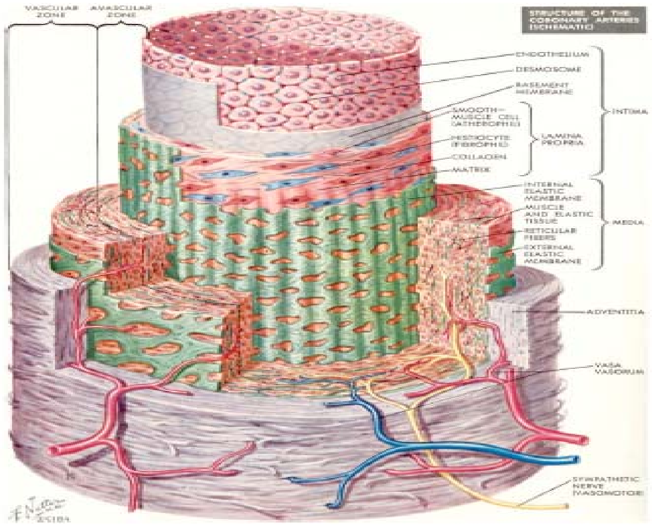


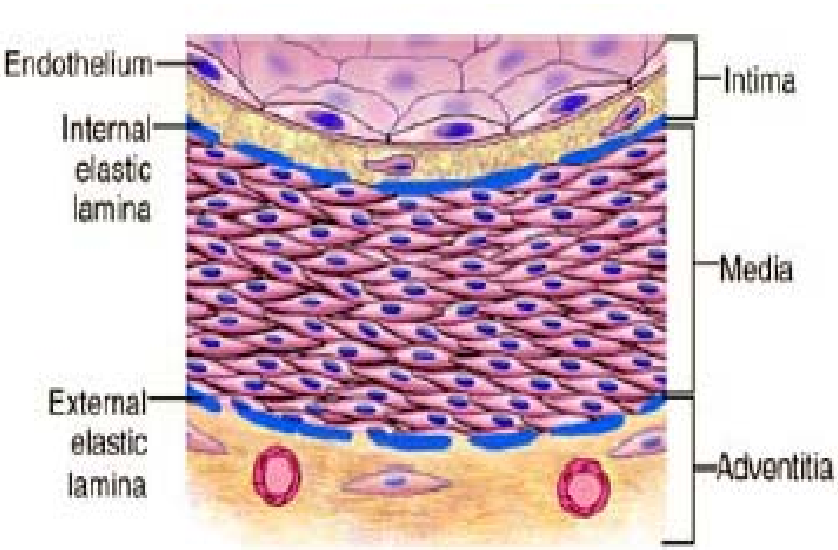




Fabrication of multilayered walls and branchy blood vessel scaffolds based on RP technology

Structure of real blood vessels





Branchy multi-layed scaffolds are required

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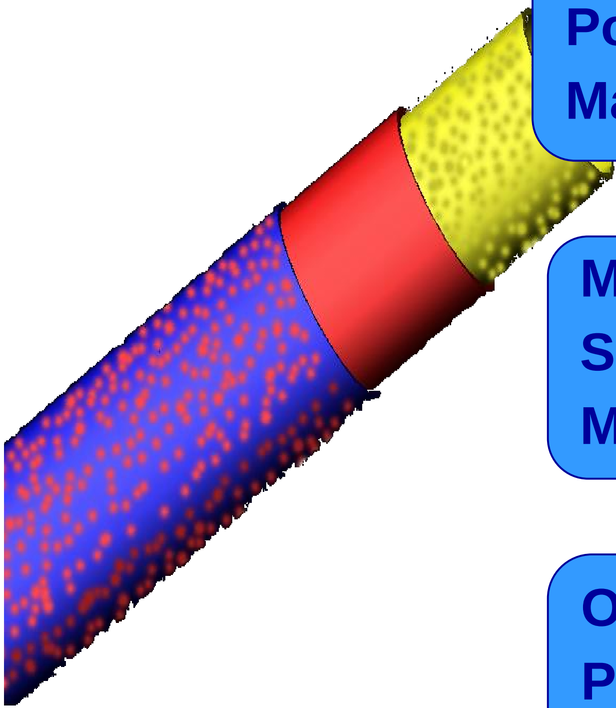
Trilayered tubular scaffolds



Inner-layer: suitable for EC
Porous structure: NaCl: 30-50 μm, M_{PLGA}:M_{NaCl}=1:9
Materials: PLGA

Mid-layer: Guarantee the mechanical performance
Solid structure: None porous
Materials: PU

Outer-layer: suitable for SMC cell
Porous structure: NaCl: 80~120 μm, M_{PLGA}:M_{NaCl}=1:9
Materials: PLGA

Making the core by MEM--RP Tech.

Dip-coating solution I

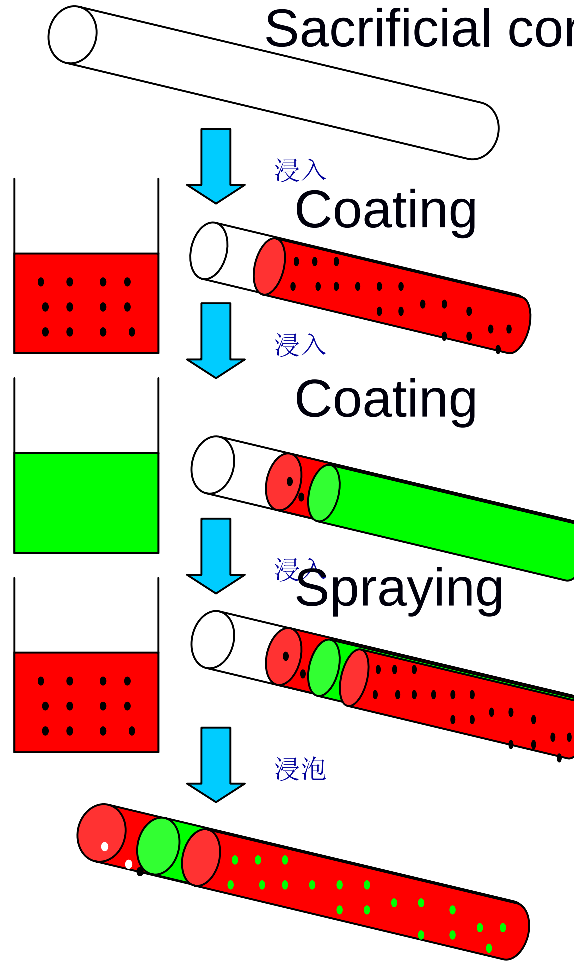
Dip-coating solution II

Spraying solution III

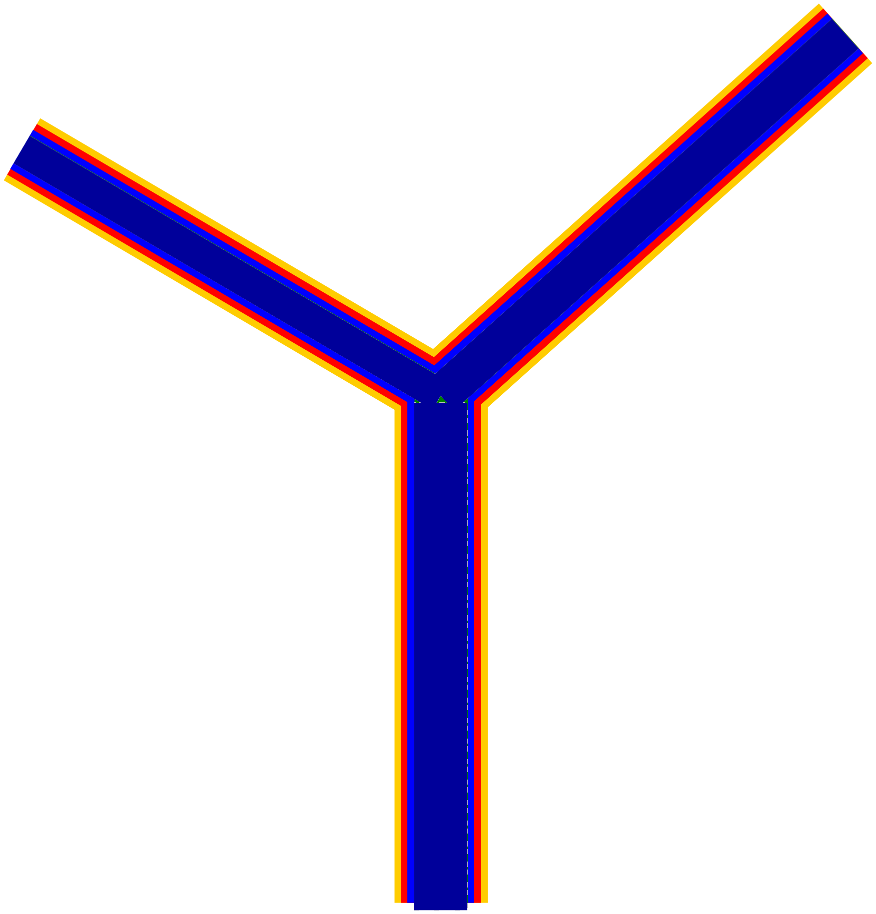
Dry in air for 24h

Leaching out the core

Dry → Scaffolds



Process of RP-Coating Technology



Design the sacrificial core

↓

Fabricating the core by RP technology

↓

Depositing the first layer

↓

Depositing the second layer

↓

Depositing the third layer …

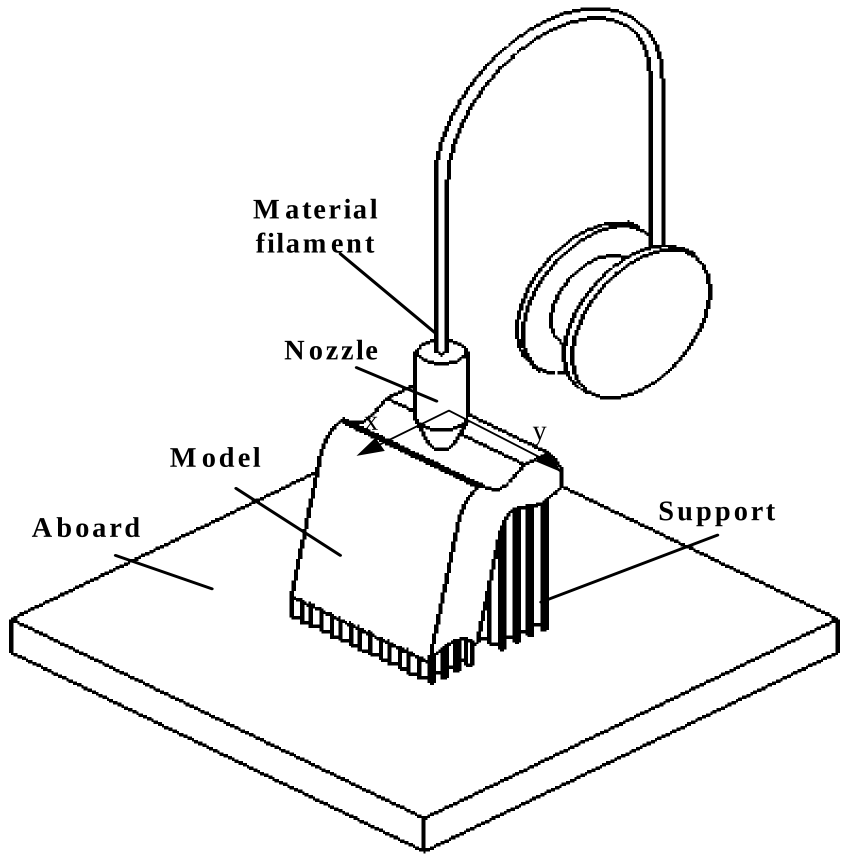
↓

Dissolve the core by water

Melted Extrusion Manufacturing

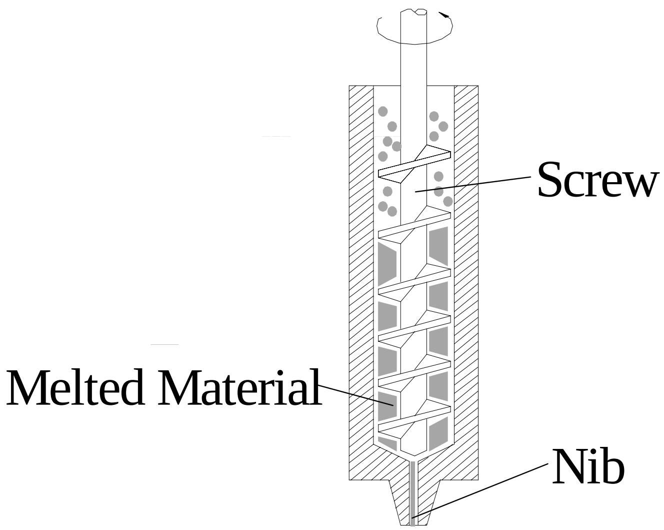
Characteristics of MEM

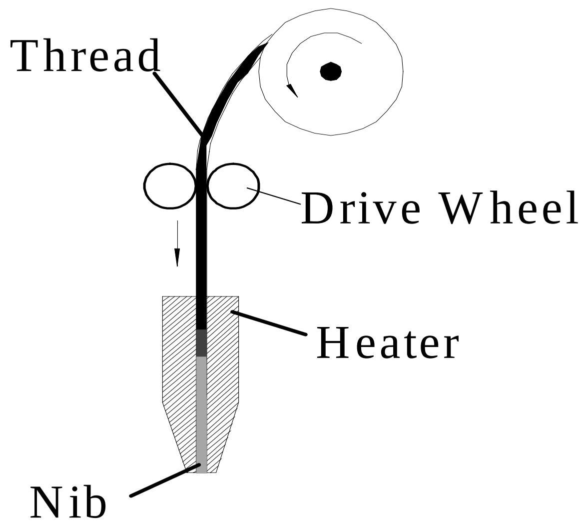
- Wide adaptability in materials
- Excellent performance
- Excellent accuracy
- Low operating cost

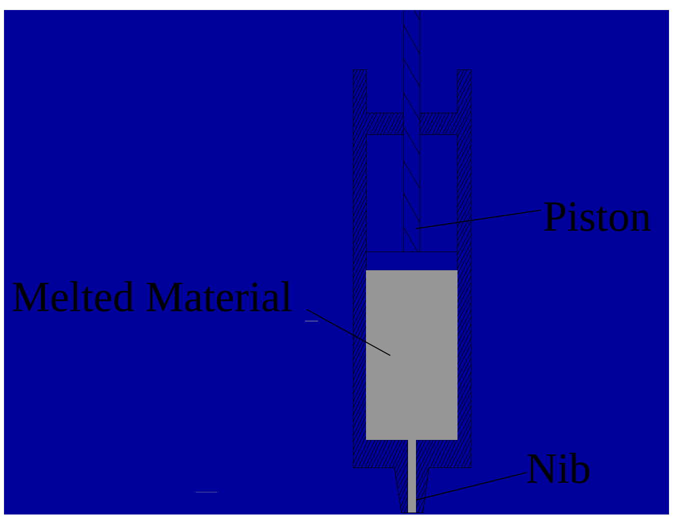


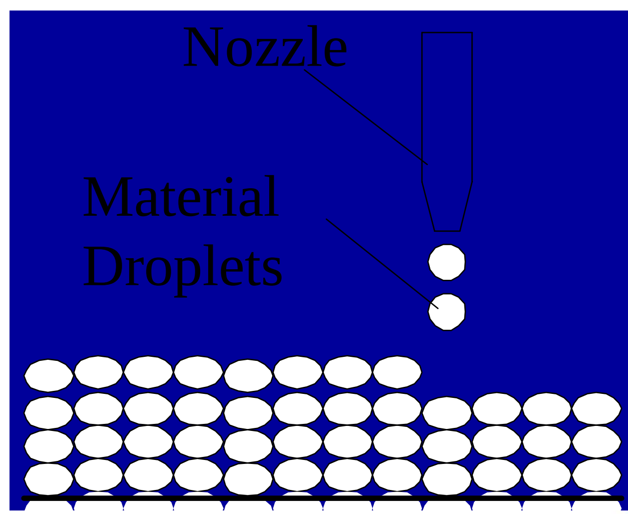
Sketch of MEM technology

Different sprayer structures in MEM









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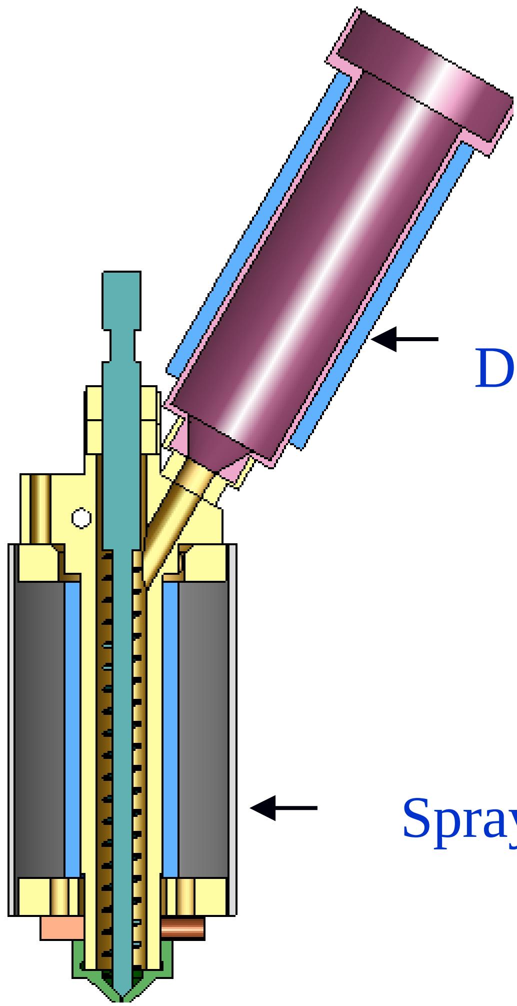
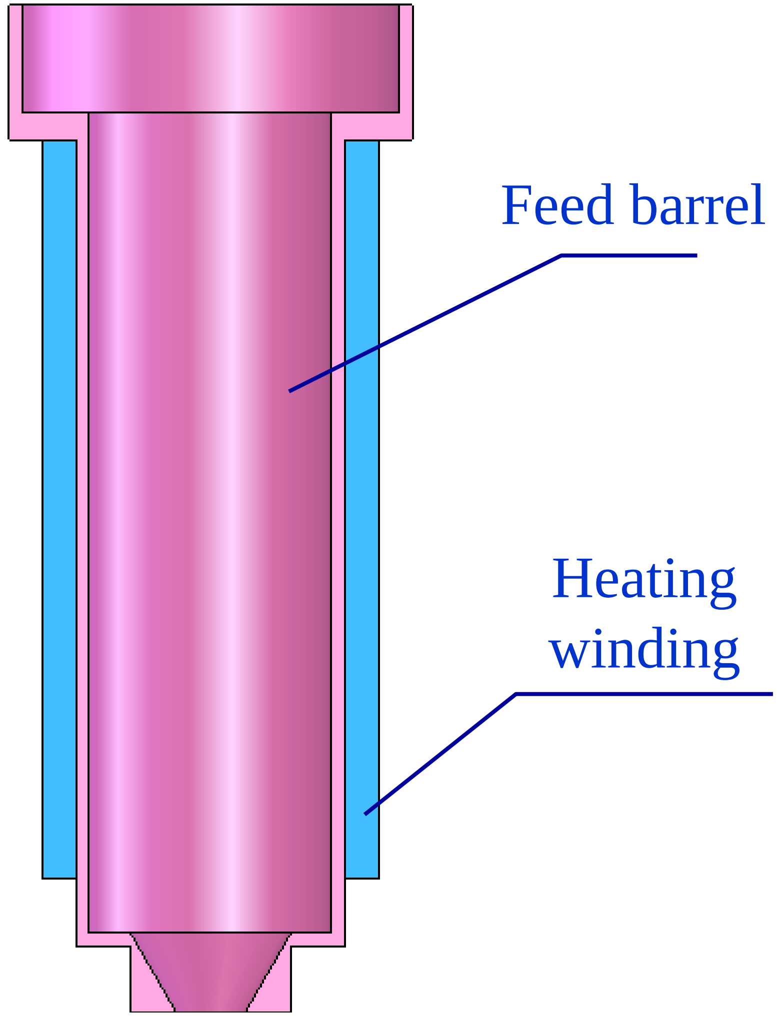


Sketch of the sprayer



Sketch of the delivery system

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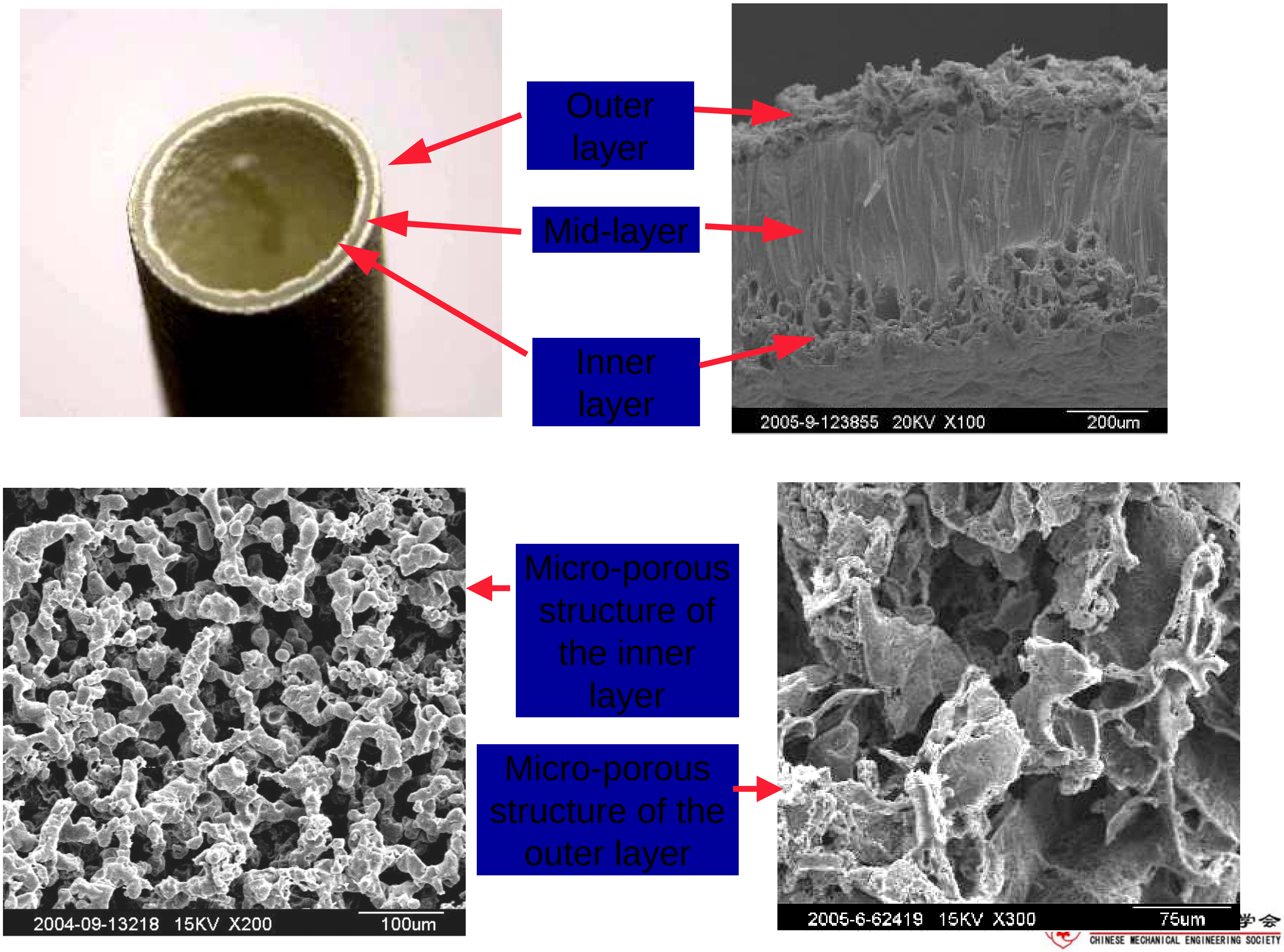

Outer layer
Mid-layer
Inner layer
2005-9-123855 20KV X100 200um
Micro-porous structure of the inner layer
Micro-porous structure of the outer layer
2004-09-13218 15KV X200 100um
2005-6-62419 15KV X300 75um
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Biological evaluation

- Biological evaluation— cultured in an pulse bioreactor
- **evaluation in vivo**

Animal model of the construction of vascularized liver histioids

- Animal model：Scid mouse；Cut off ¾ of the mouse's liver; Implant the scaffold into the abdominal cavity of the Scid mouse

- take out the sample one month later after the implant

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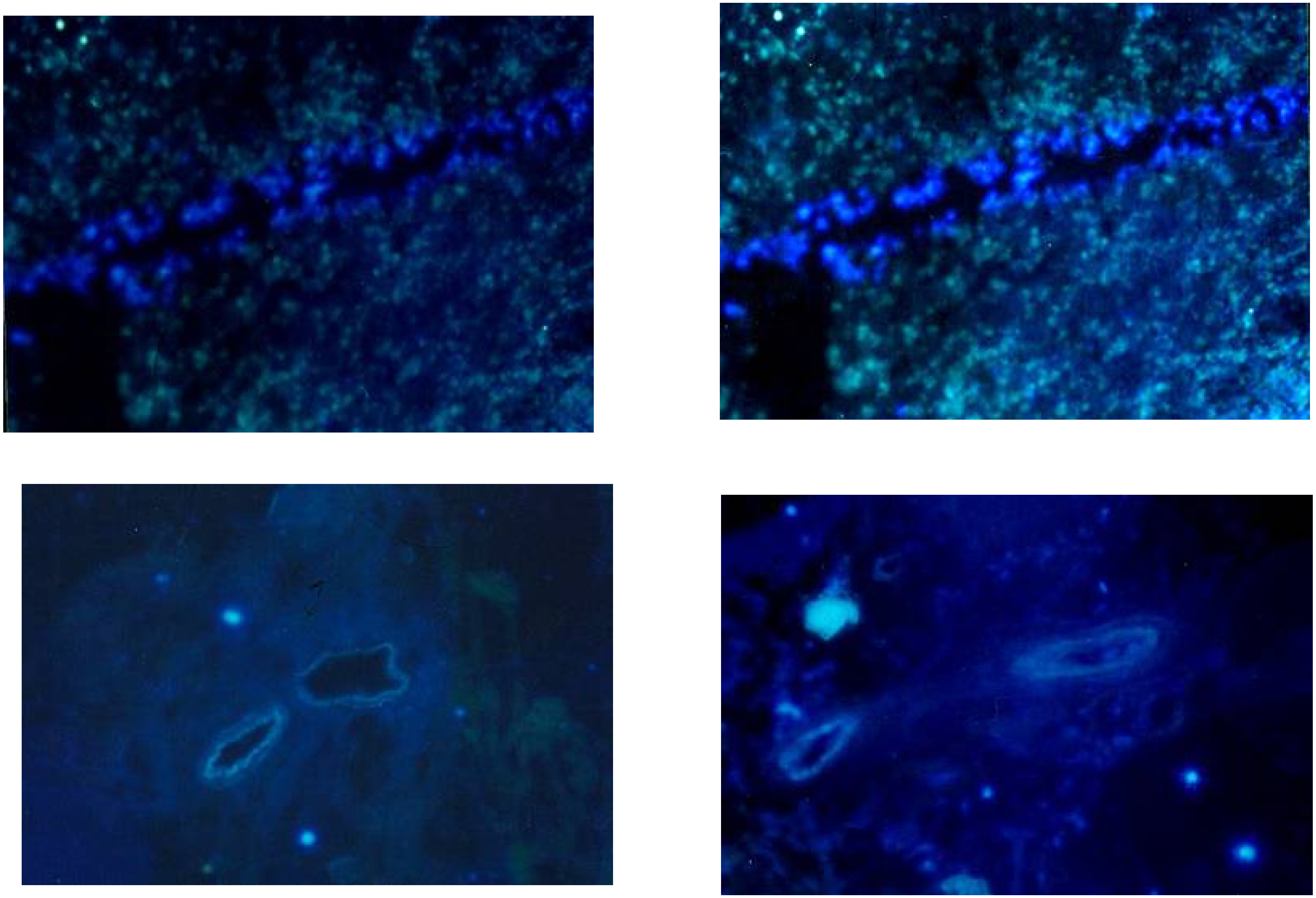

Capillary-blood-vessel-like tissue

100X

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dilemmas

The challenges of Tissue Engineering

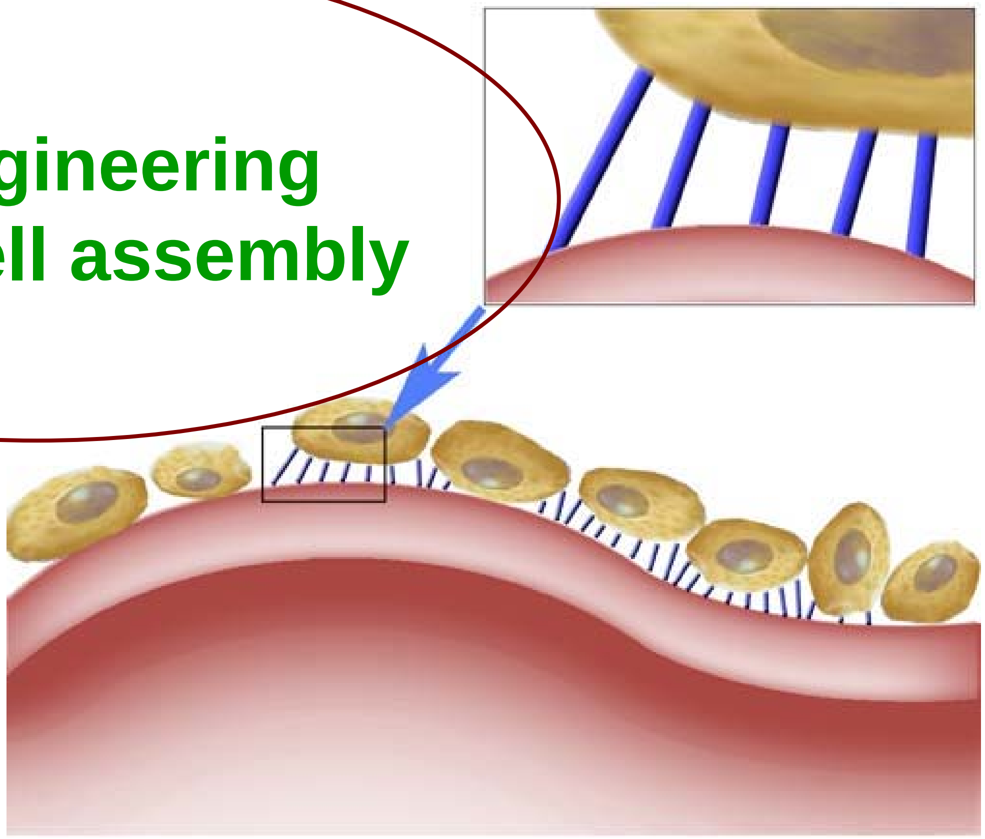
(1) It's difficult for cells to adhere and proliferate, because of thick scaffold.

(2) Most of organ or tissue contain kinds of cells, which would be a big challenge for current technology to arrange diverse cells in place.

(3) The limited cell adhering density.

(4) The vascularization is limited.

Tissue Engineering
Indirect cell assembly

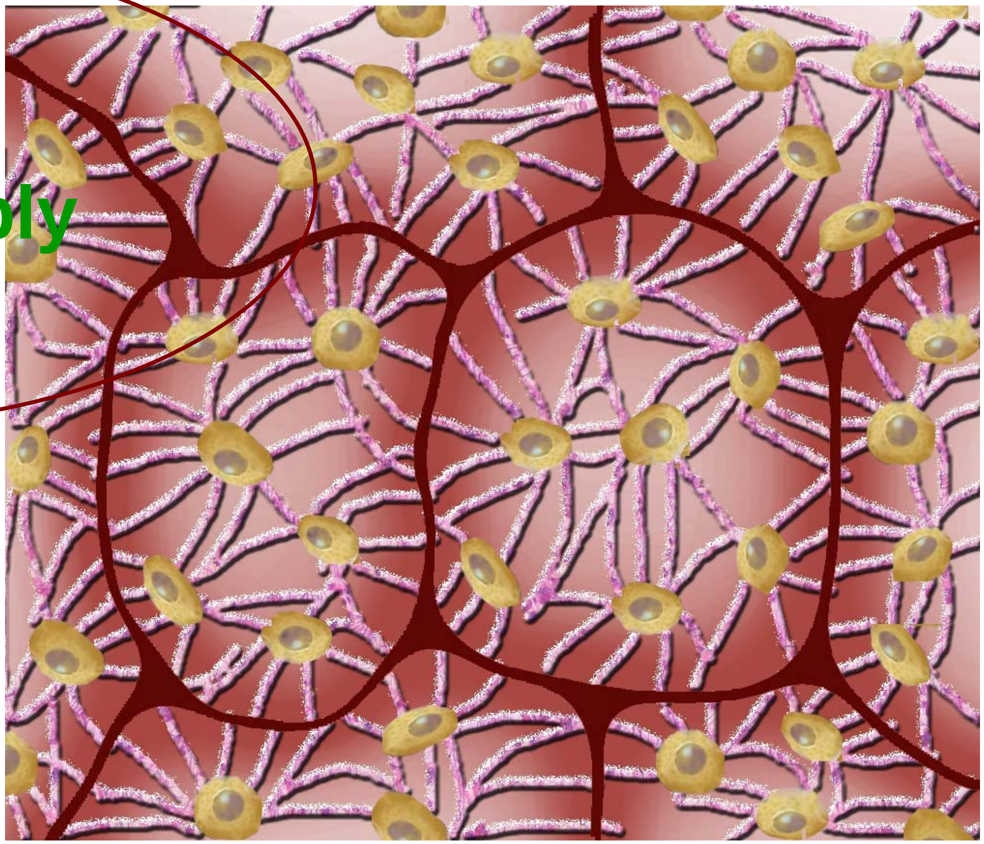


The cells reside on the surface of the matrix.

(1) Cells reside in an ansymmetry growth environment, not resembling the natural 3D status

(2) The essence problem have not been changed through modification of scaffold surface only

Cell assembly
Direct cell assembly



The cells reside in the three dimensional structure, greatly resemble the natural condition, every cell has equality opportunity to growth and communicate with each other.

Dr. Robert F. Service:

Tissues such as the kidney and lung….. consist of numerous cell types that must be arranged in the proper three dimensional structure and coaxed to express particular genes at different time.

Structure tissue such bone and cartilage are not as complex.

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The research and rebuild of the microenvironments cell lived from

"on the surface" to

"in the structure " produce a series of essence changes.

- **Biomanufacturing and organism manufacturing**
- **Tissue engineering, the rich fruits and the dilemmas**
- **Direct cell controlled assembly**
- **Practice the cell controlled assembly technology**

Three-dimensional Controlled Cell Assembly

With the control of computer, diverse cells and ECMs are deposited into a designed, special and proper spatial structure, to form a structure (analogy tissue precursor). After training and culturing (three dimensional), this structure grows into a tissue and/or organ with certain physiology and mechanical function.

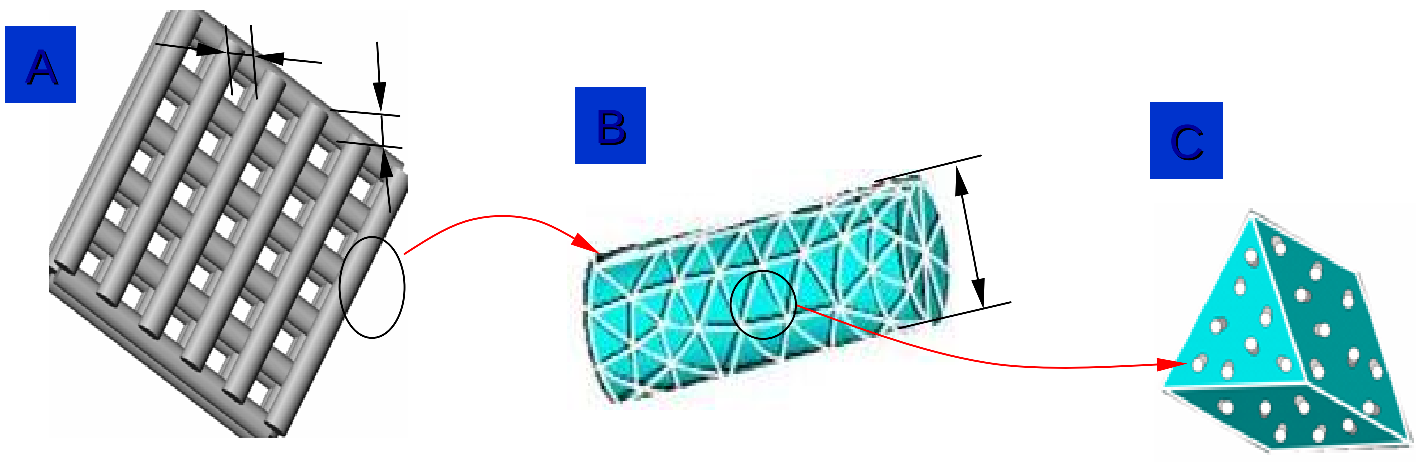
The basic requirements of cell controlled assembly

- The constructed three dimensional analogy tissue precursor (ATP) have the ability that the interior cells could take in enough nutrients and oxygen or get rid of sufficient carbon dioxide to continue growing.
- ATP could sustain long-term stabilization.
- The assembled amount of cells could be controlled.
- The predefined size and shape could be formed easily.

- **From the macro point of view, the cells or the cell cluster has been arranged corresponding to the anatomical model and formed a whole structure.**

- **From the micro point of view, cells may be restricted in some degree, but not fixed completely, so it could be ensured that the communication, connection , growth and various metabolizing between cells.**

Hiberarchy structure

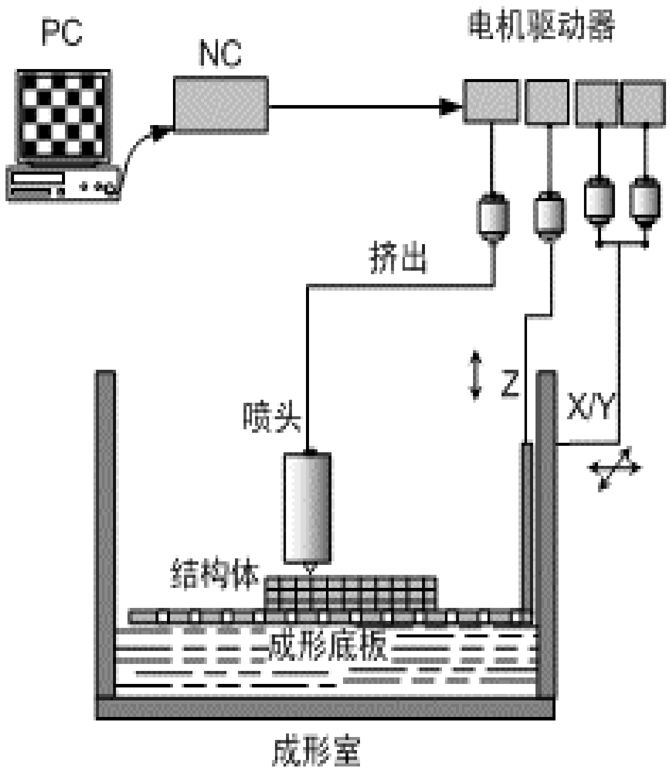


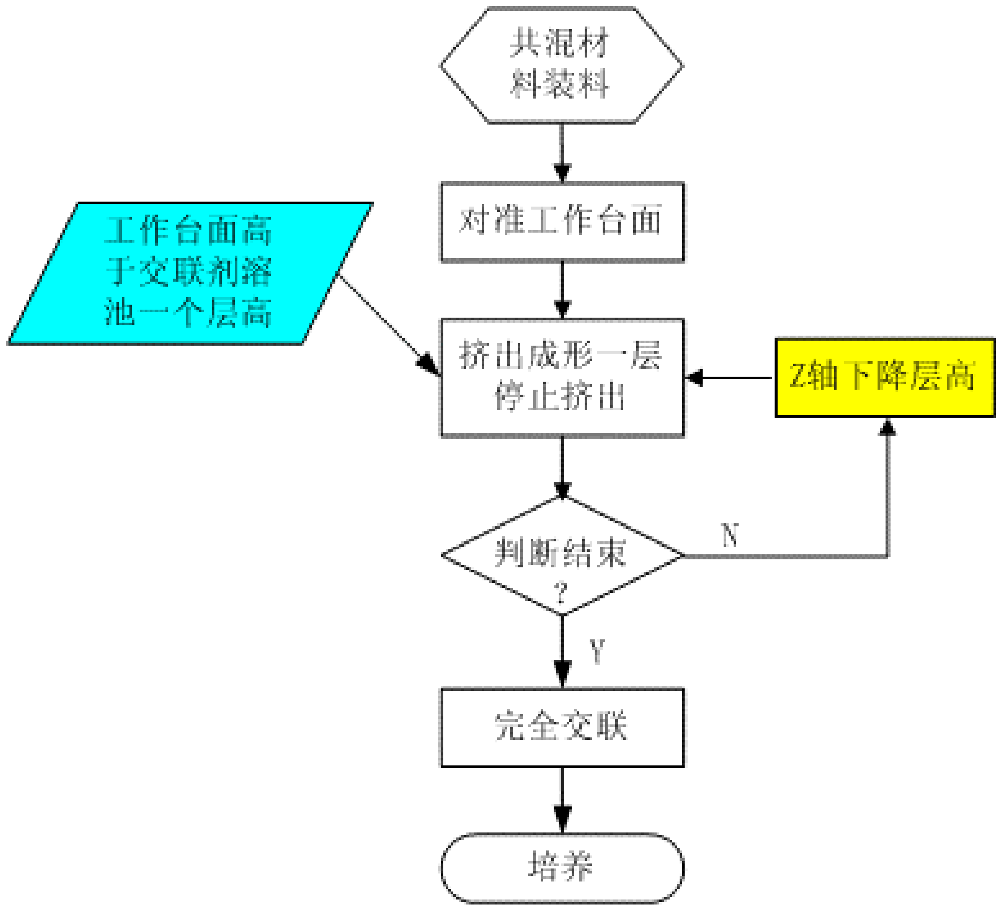
The figure illustrates the three main structural levels of a hiberarchy structure we promoted.

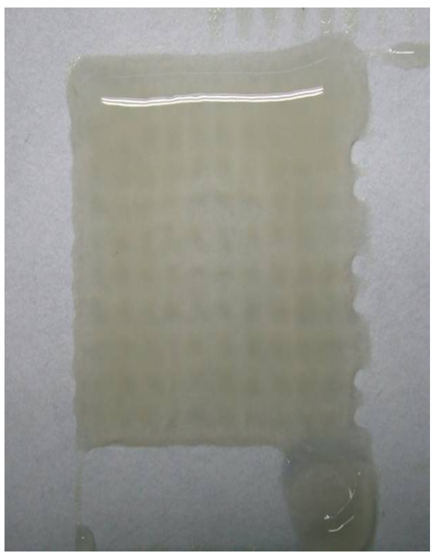
- **Figure A shows the tertiary structure, namely cellular structure. It is a structure with accumulated filaments that are composed of mixture of cells and hydrogels and connected channels that will serve as the vascularizing system.**
- **Figure B shows the secondary structure，namely reticular structure. Which is composed of a supporting net and micro-units.**
- **Figure C shows the primary structure (micro-unit), namely location structure. This micro-unit ensures that the inner cells of structure have enough migration space.**

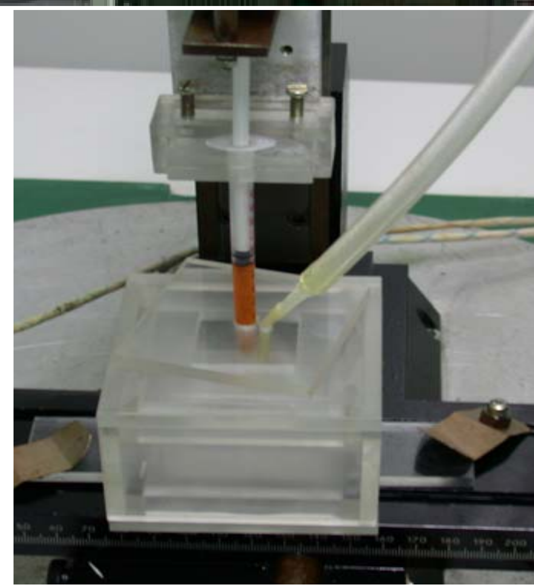
Forming process

（1）Delamination crosslink process, a RP Tech.



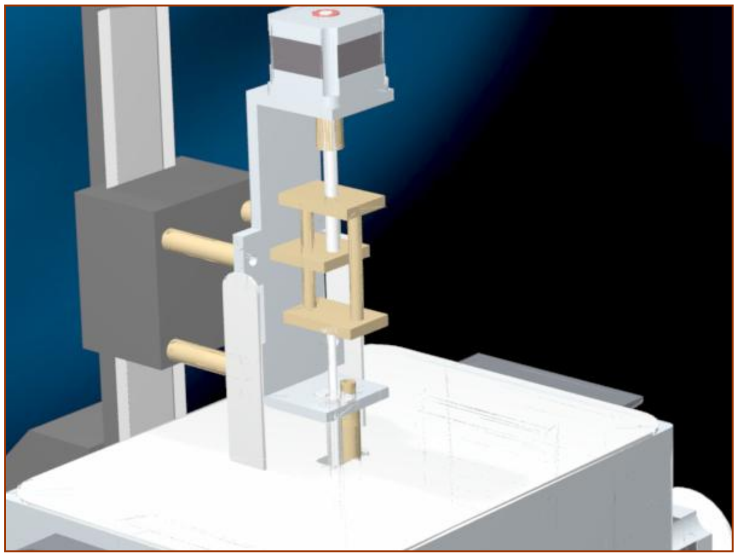






Cell controlled assembler II

core parts of assembler



Odd nozzle extruding machine
of Cell assembler I

Multi-nozzle extruding machine
of Cell assembler II

The table listed forming parameters

Extrusion cavity volume (ml)	1
Nozzle diameter (um)	200
Scanning speed (mm/s)	20
Extrusion frequency (Hz)	79
Material concentration (%)	5
Cross linker concentration (%)	6
Lattice size (mm)	0.8

The experimented cells

cartilage cell (软骨细胞)

fibroblast cell (纤维细胞)

hepatocyte cell (肝细胞)

endothelium cell (内皮细胞)

myocardiac cell (心肌细胞)

hepatocyte + fibroblast

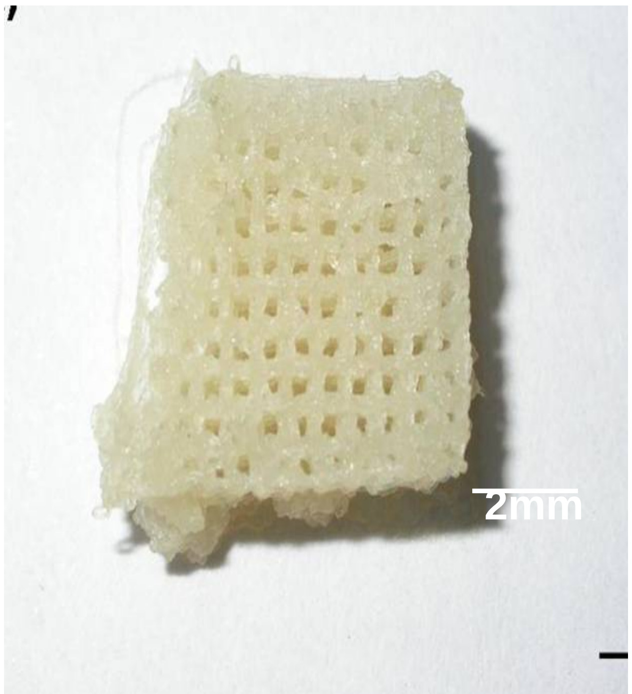
The experimented materials

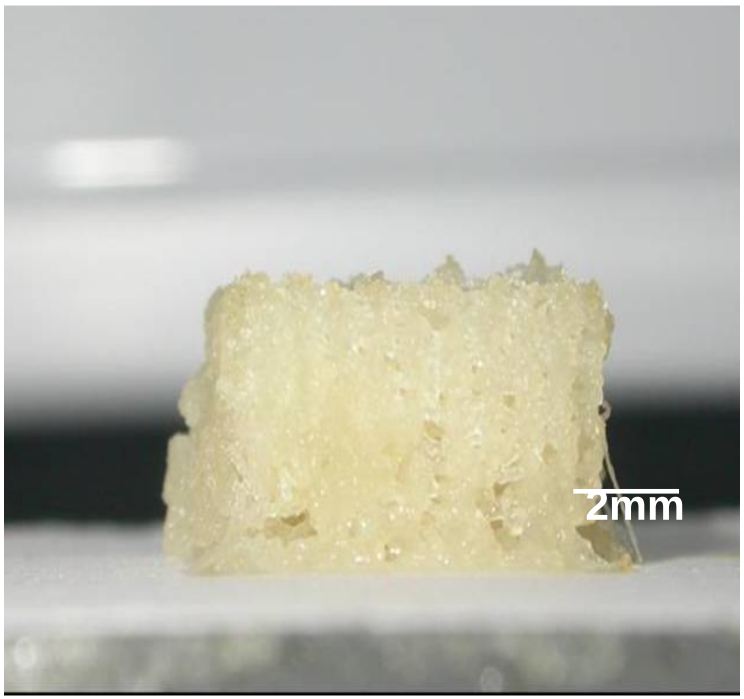
gelatin (凝胶)

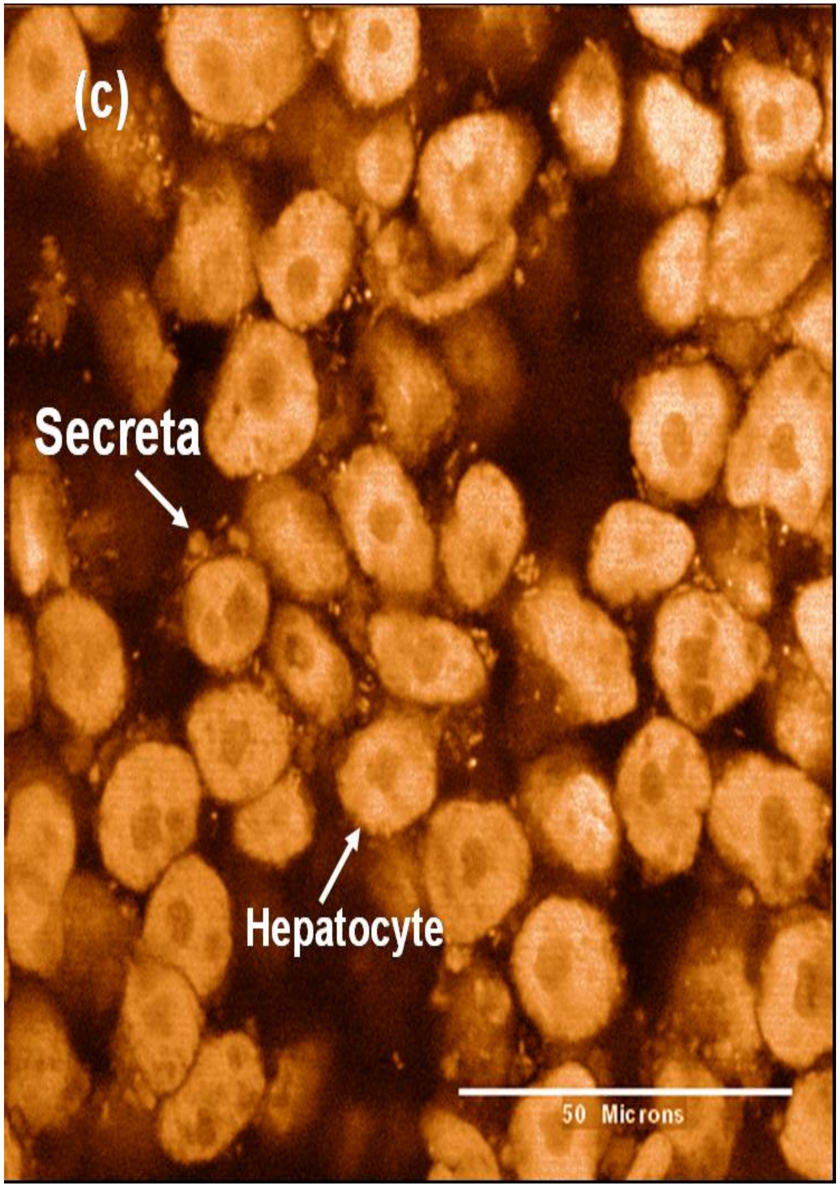
sodium alginate (海藻酸钠)

chitosan (壳聚糖)

3D structure with hepatocyte/gelatin







Confocal laser scanning (CLS) image of the hepatocytes
One week after *in vitro* culture, stained with propidium iodide (PI,sigma USA)

Hepatic cells initially resided in the micro-environment provided by the 3D formed structure and presented large and round shape

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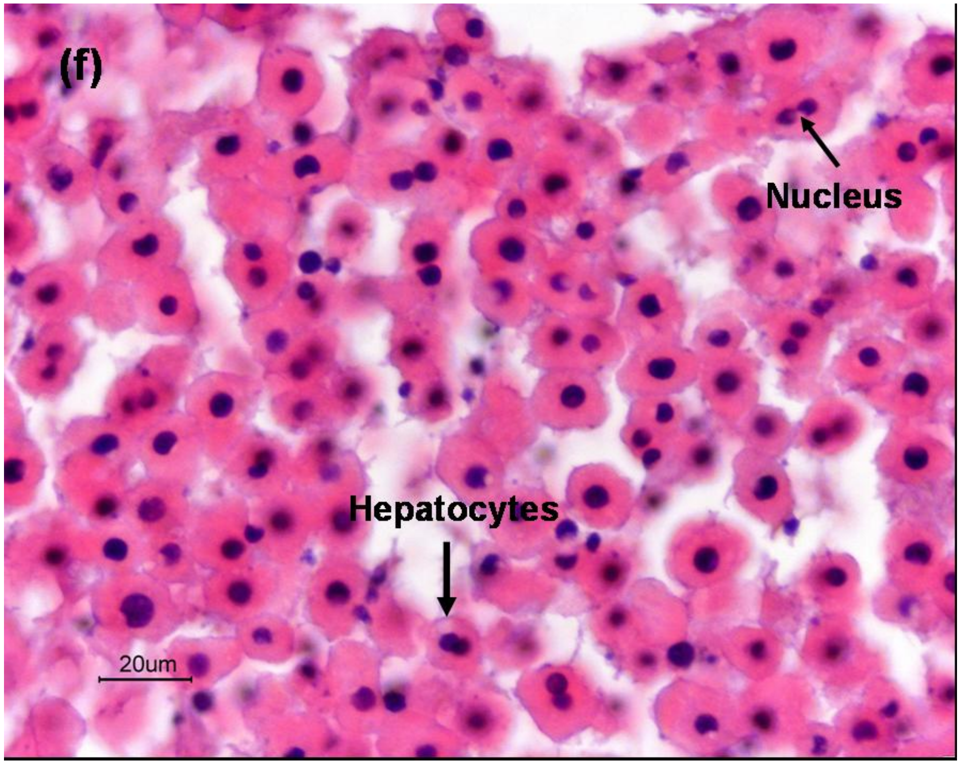

Image of histological section after two weeks in vitro, hepatic cells were still surviving and proliferating vigorously everywhere in the 3D structure, the long sinusoids were observed in many fields, as shown in picture.

Conclusion

- The 3D structure plays a significant role of maintaining the long lasting viability and function.
- through changing the spatial position and releaseing amount of grow factors to research the corresponding function of grow factors and signal molecules delivered into a certain position.

The current hepatocytes researches have revealed the great potential in the 3D cultivation system of stem cell.

It may play an important role in research of culturing some specified cells and/or tissues for transplants, the differentiation mechanism of stem cell and actually the development of human body.

The structure made, which could be viewed as an open system and bear an analogy with natural tissue, so it would be an efficient platform to design many physiological tissue model in vitro for the research of pharmaceutical(制药学) analysis and pathogenesis(发病机理).

An International Conference on Bio-Manufacturing Technology, held 2008

- A International Symposium on Bio-Manufacturing Technology was held in 2005,organized by Tsinghua Univ, sponsored by National Foundation for Science of China and US, and our society
- 3rd International Conference on Rapid Prototyping and Manufacturing, (ICRPM '2008 Beijing) Beijing, China October 18 & 20 2008

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Topics

- Virtual Prototyping
- New trends on RPM technologies and machines
- Development of rapid prototyping and rapid product
- Rapid tooling
- Bio-manufacturing

- http://me.tsinghua.edu.cn for more Information
- Global Alliance Rapid Prototyping Association' annual meeting will be held in the same time

- **Thanks for Prof. Yongnian Yan and his multi-disceplinary team to offer me his updated information**
- **Thanks for your attention**

- **EC：endothelial cell 内皮细胞**
- **PLGA：poly(lactic acid-co-glycolic acid) 乳酸与乙醇酸共聚物**
- **PU：polyurethane 聚氨酯**
- **SMC：smooth muscle cell 平滑肌细胞**
- **Dip-coating浸蘸涂覆**
- **ECM：extracellular matrix 细胞外基质成分**
- 颜

- **Department of Mechanical Engineering**
- **Tsinghua University**
- **Beijing 100084**
- **R.P.CHINA**
- **8610-62783565**