1. FFF Techuologies,FFF~Scaffold Mnuf.
2. Scaffold Manuf. Technologies
3. Non-degradation Scaffold
4. BONE Tissue Eng. Scaffolds
5. 3-D cell Assembled
6. Laser Directed Guided Writing(LDGW) of cell

BONE

Tissue Scaffolds

(Degradable)

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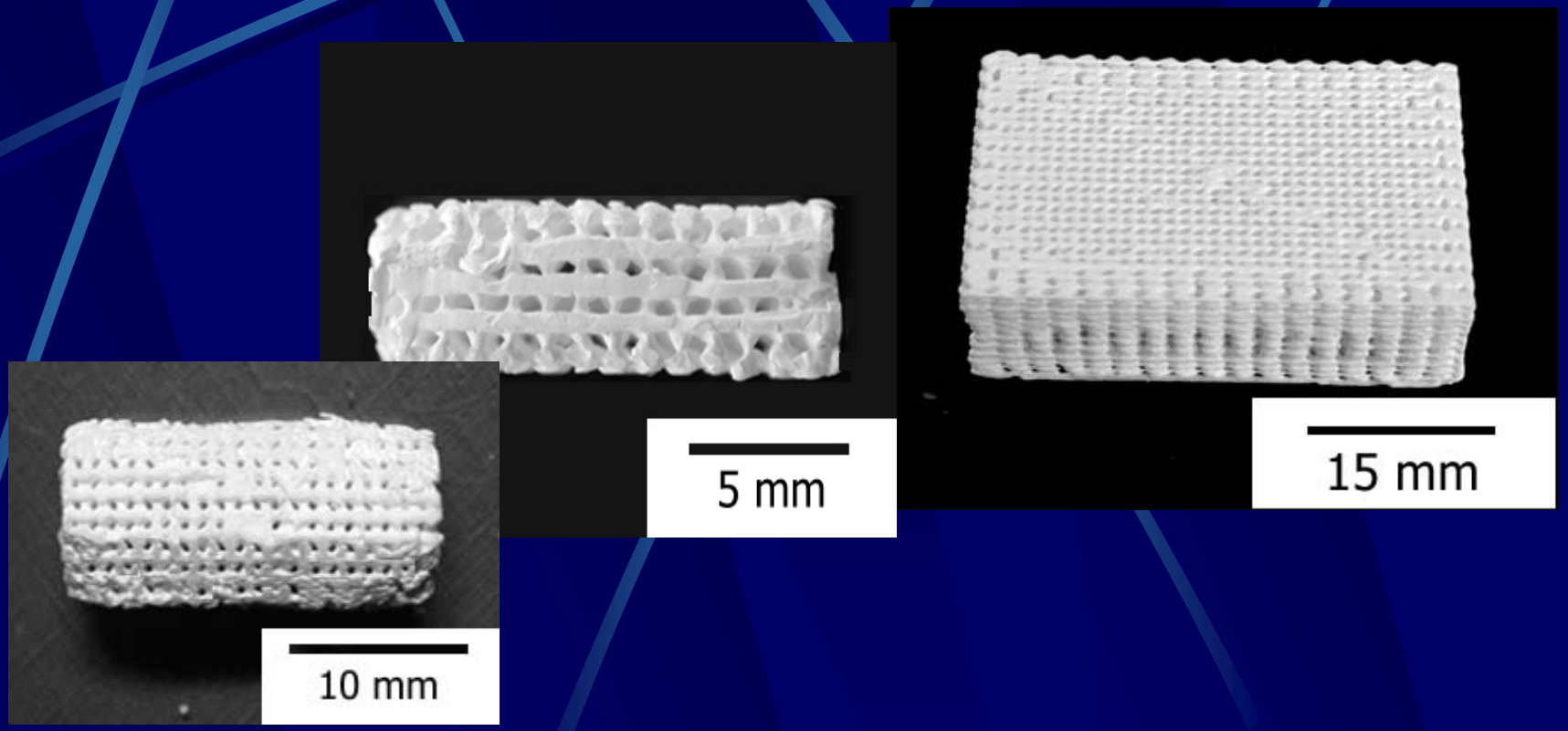
Scaffold poly (L-lactic acid)
Tricalcium Phosphate



Developed in CLRF, Tsinghua University

Figure by Tsinghua University, CLRF&CBM

Implant bone Tissue Scaffold

Dog

Figure by Tsinghua University, CLRF&CBM

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No Scaffold

Dog

Figure by Tsinghua University, CLRF&CBM

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- Deeply integrating *manufacturing science* with *life science* and *cell biology*, viewing cells and the extra-cellular materials as assemble units

- we propose the 3D controlled assembling by *FFF* to manufacture the Analogy Tissue Precursors (ATPs).

Analogy Tissue Precursor ——

The 3D structure with the

characteristic of living and metabolism

Illustration of the organism manufacturing

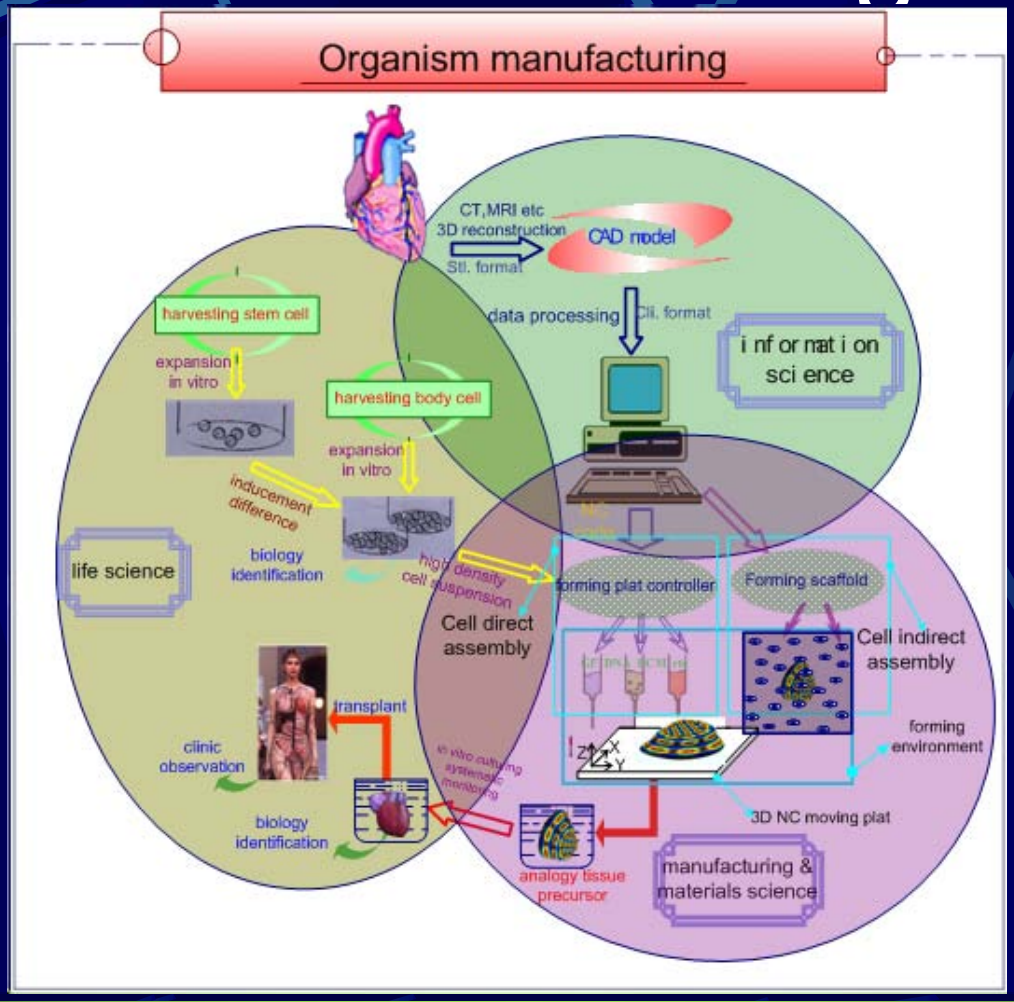


Figure by Tsinghua University, CLRF&CBM

Two photos removed for copyright reasons.

Bioplotter

Landers&Mulhaupt(2000)

EnvisionTec

Cell printer

Vladimir Mironov, et al.,"Organ printing : computer-aided jet-based 3D tissue engineering",Biotechnology,Vol.21 No.4, April 2003

Cell controlled assembler I

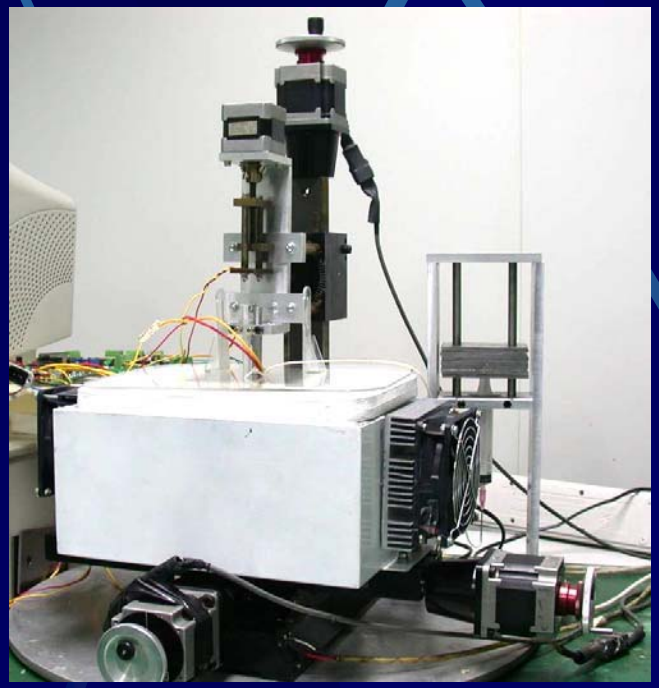
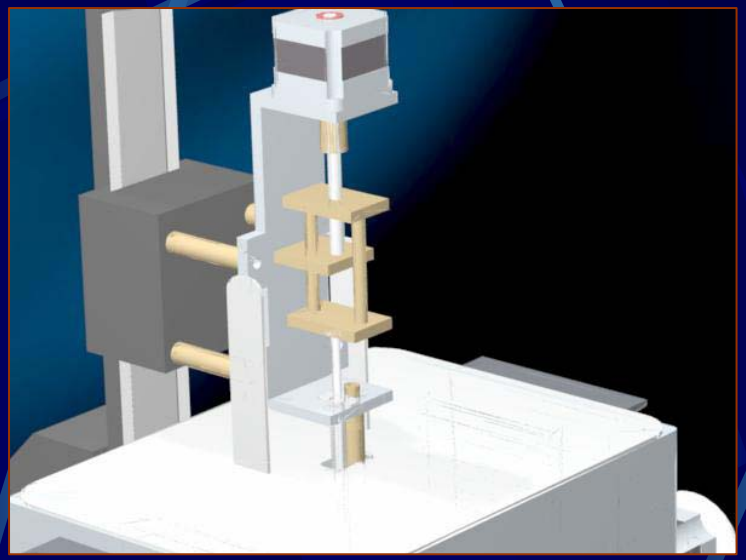


Figure by Tsinghua University, CLRF&CBM

core parts of assembler



Odd nozzle extruding machine of Cell assembler I

Multi-nozzle extruding machine of Cell assembler II

Figure by Tsinghua University, CLRF&CBM

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
  - fibroblast cell
  - hepatocyte cell
  - endothelium cell
  - myocardiac cell
  - hepatocyte + fibroblast
- The experimented materials
  - gelatin
  - sodium alginate
  - chitosan

3D structure with hepatocyte/gelatin

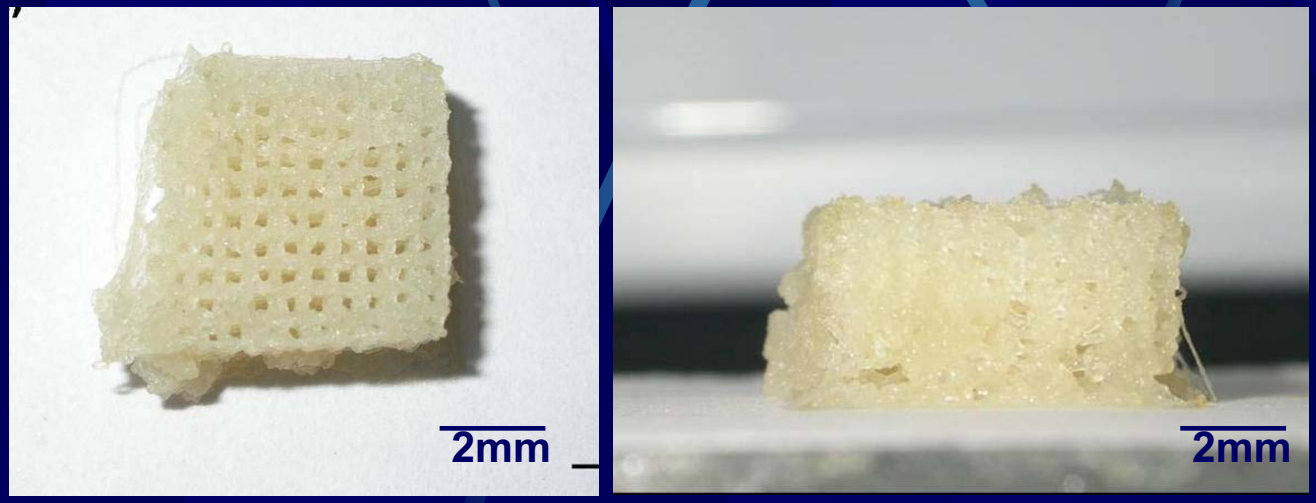
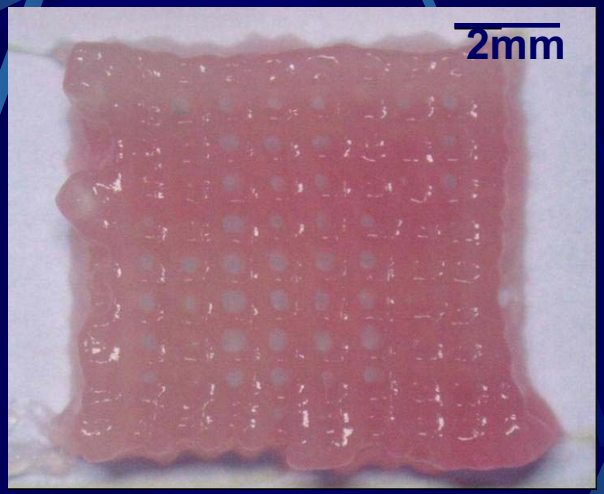
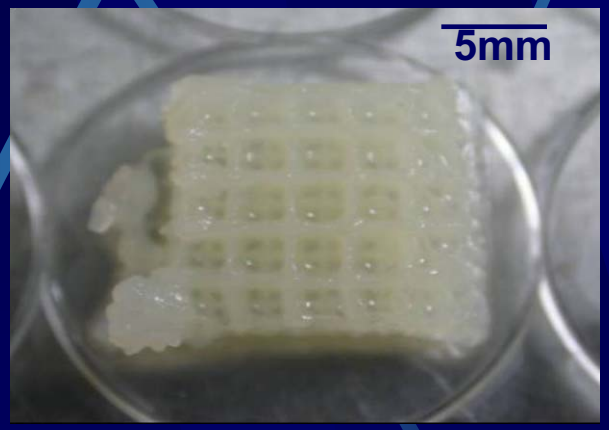
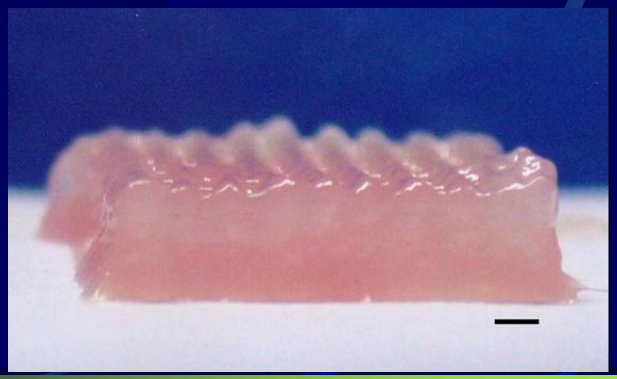


Figure by Tsinghua University, CLRF&CBM

3D structure with hepatocyte/gelatin/sodium alginate







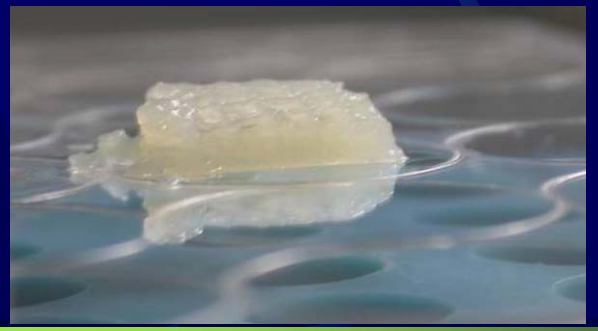
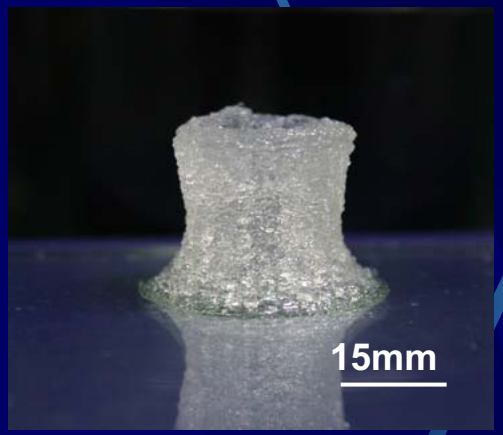
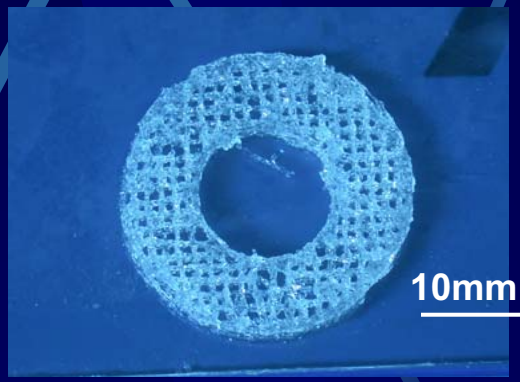


Figure by Tsinghua University, CLRF&CBM

3D structure with chitosan





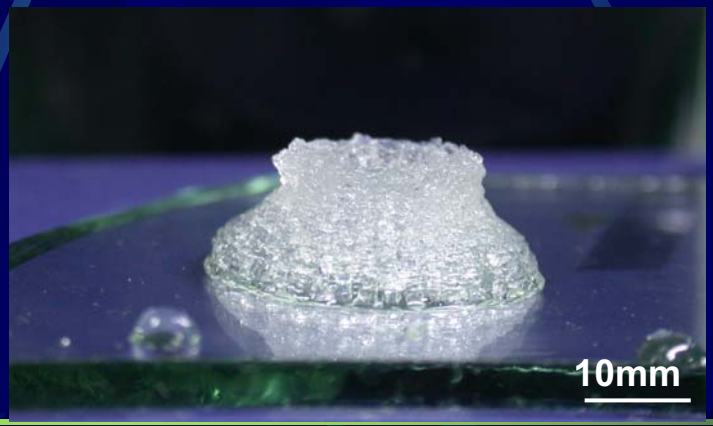
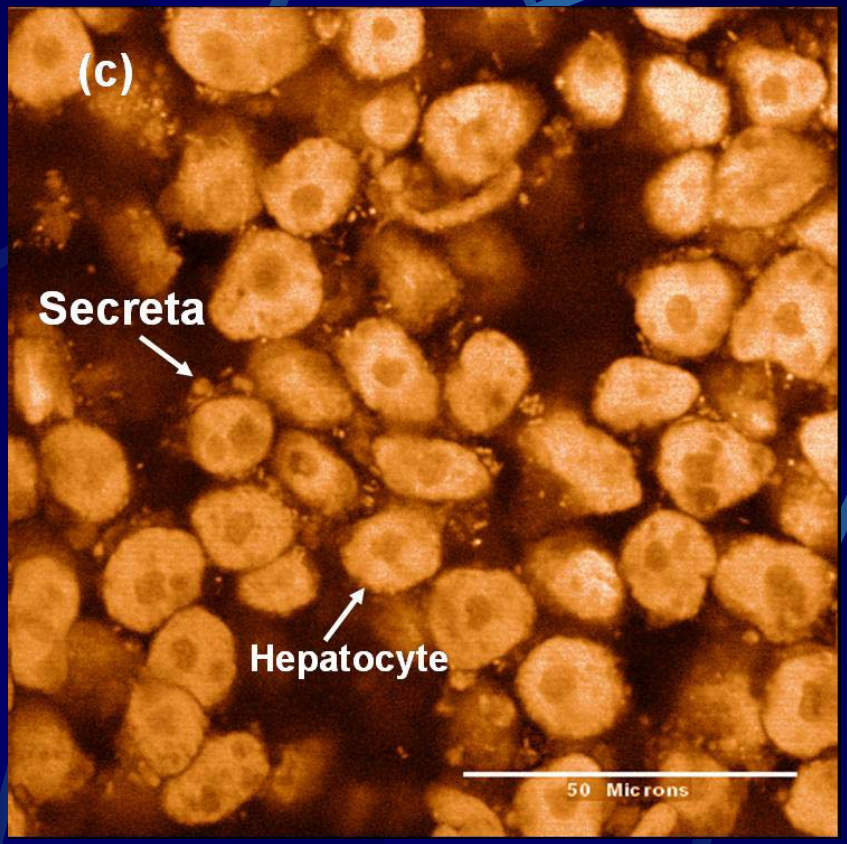


Figure by Tsinghua University, CLRF&CBM



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

Figure by Tsinghua University, CLRF&CBM

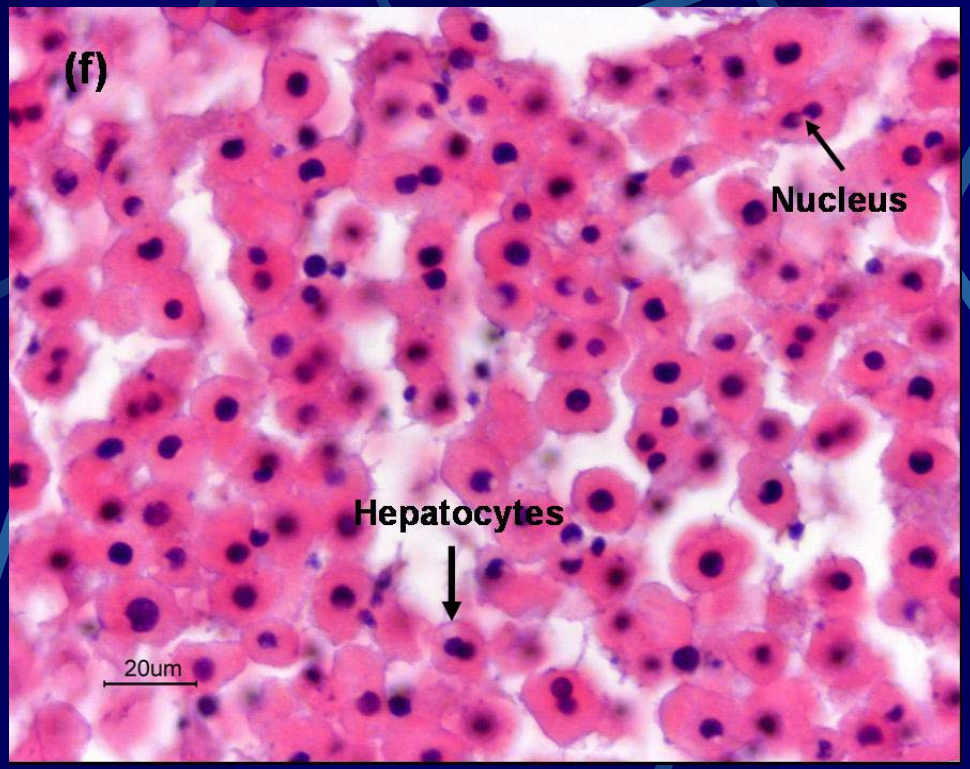


Figure by Tsinghua University, CLRF&CBM

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.

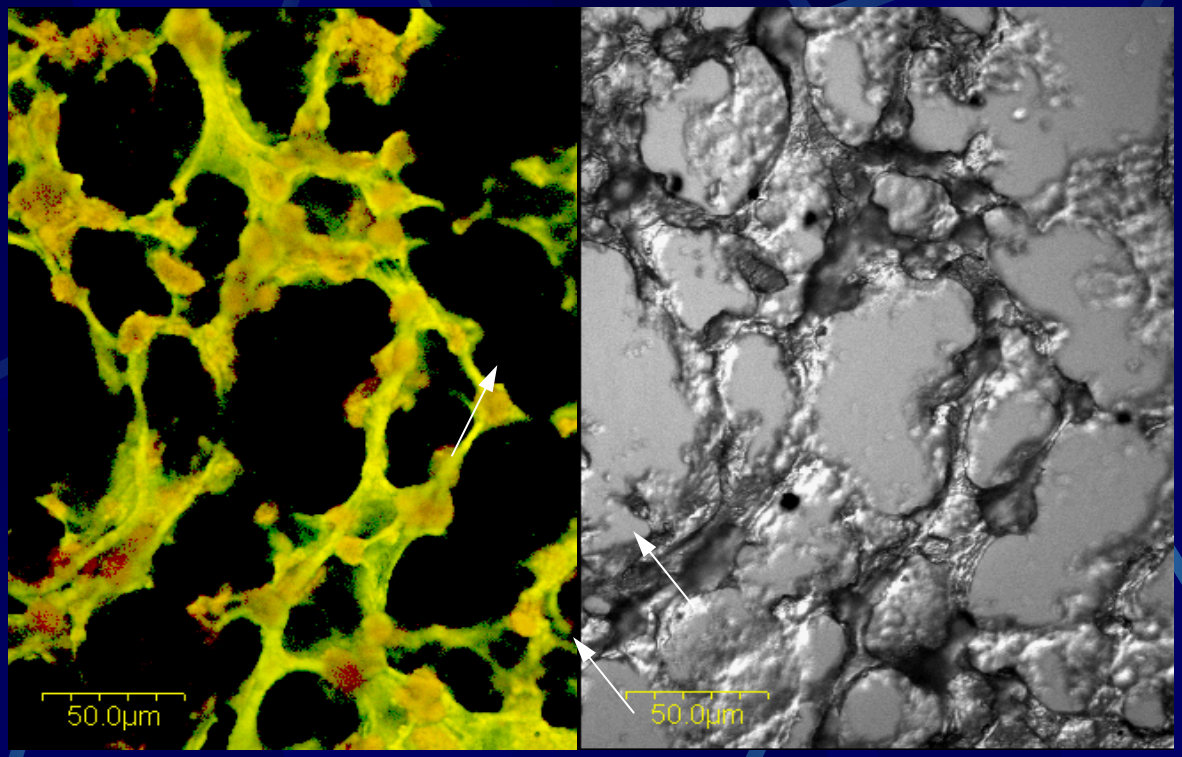


Figure by
Tsinghua University,
CLRF&CBM

LSC images of the hepatocytes after three weeks culture.
a) LSC observation with both PI staining and FITC-conjugation.
b) Negative control.
The cells displayed positive for albumin antigen-antibody reaction, and negative for the negative control of abnormal rabbit serum.
Arrows indicate the duct-like structures were formed.

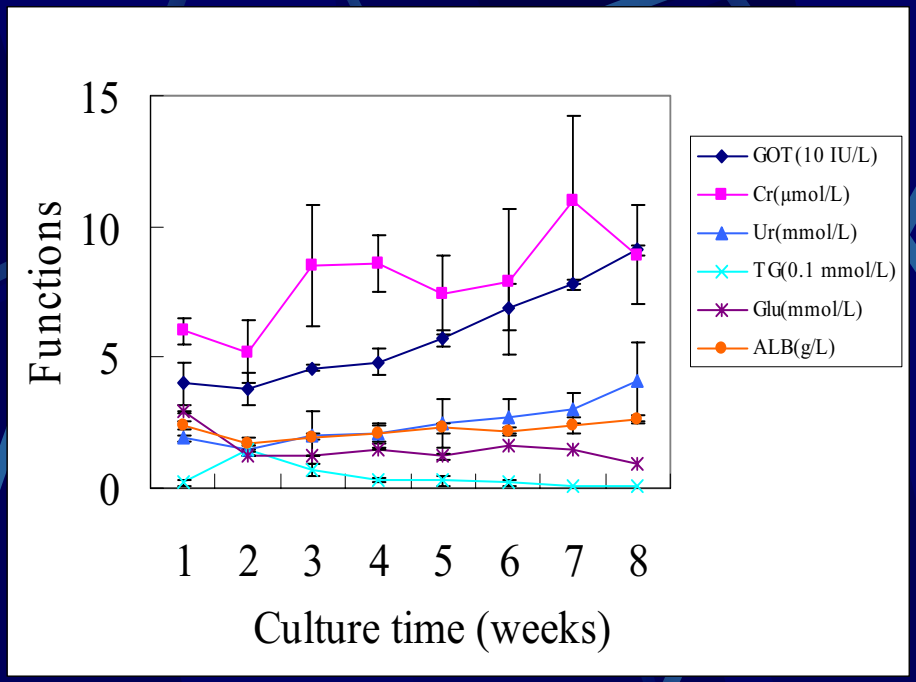


Figure by Tsinghua University, CLRF&CBM

The amounts of albumin secretory and urea synthesis increase during 8 weeks culture.

The amounts of albumin and the urea were in relative low level at the first 3 weeks, then increase in 3 to 6 weeks. After 6 weeks, the amounts kept in high level consistently. It indicates that hepatocytes perform liver-specific function in the network block.

- Forming process:

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Principle of Laser Guided Direct Writing (LGDW)

First posed by Renn, Michigan Institute of Technology, USA

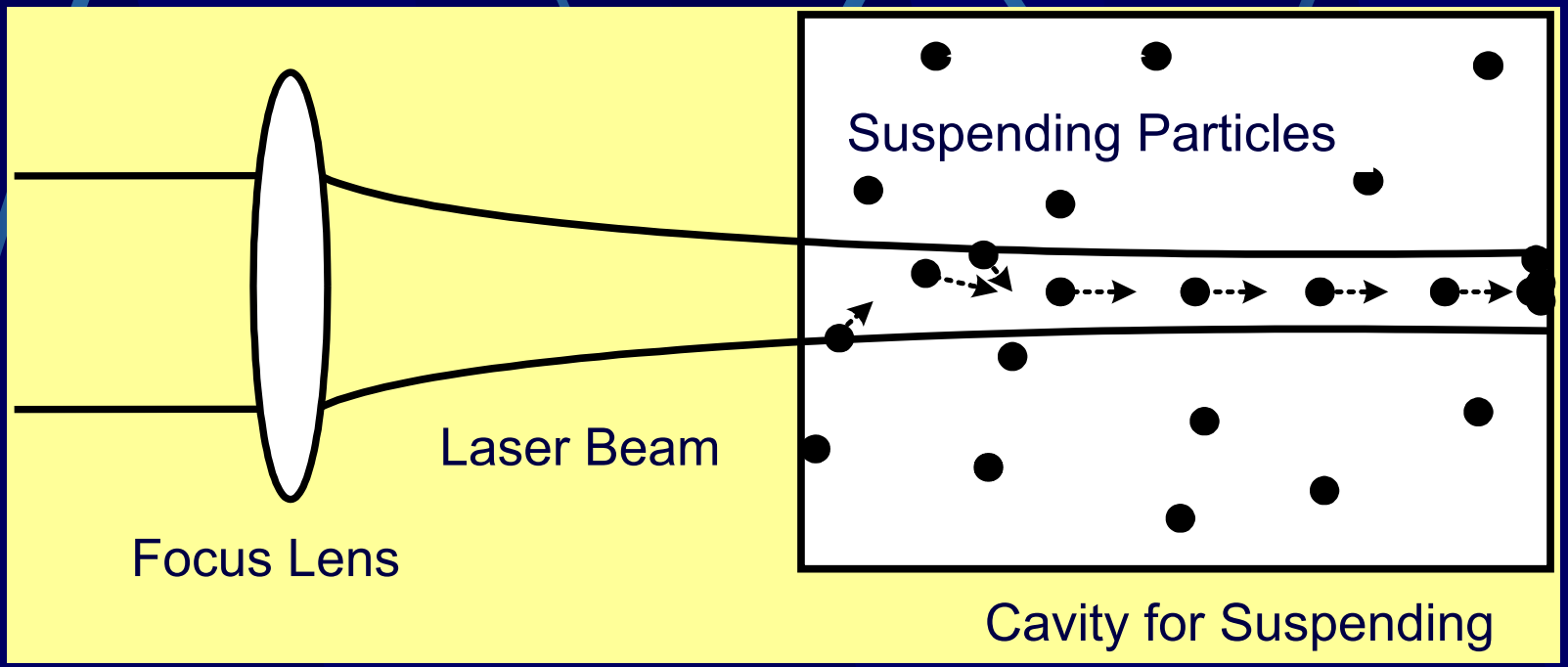

Figure by Tsinghua University, CLRF&CBM

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Influences of the medium on LGDW

(1) flotage

(2) disturb of convection

(3) attenuation of the light power

The practice system

Figure by Tsinghua University, CLRF&CBM

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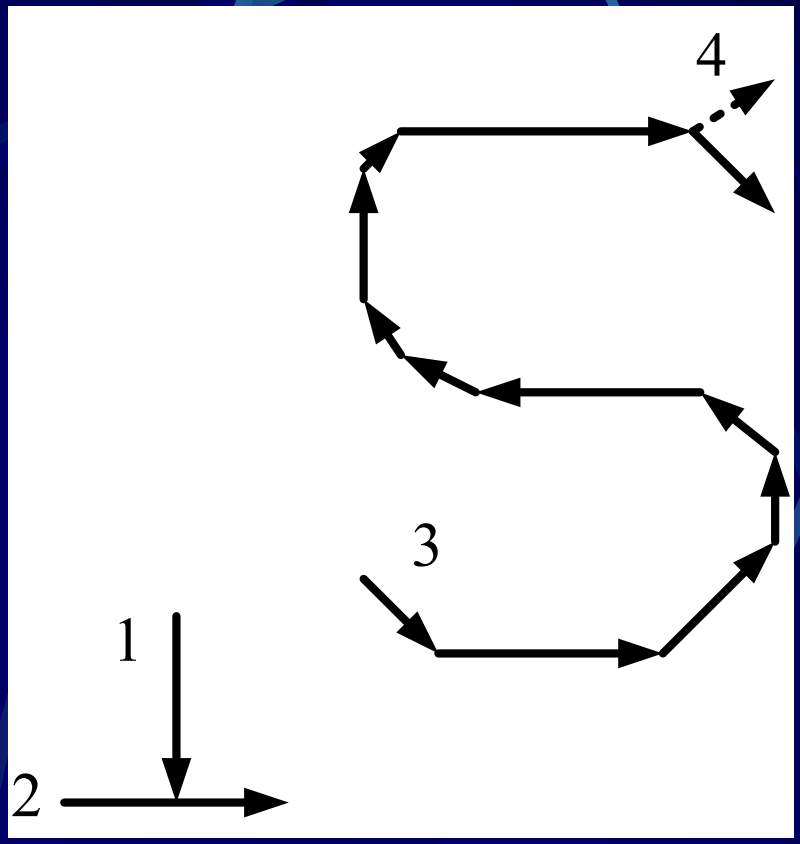
Laser Power 500mW，girdling radius 15um
Spacing between two dots
10~15um，Deposition time: 10min/dot



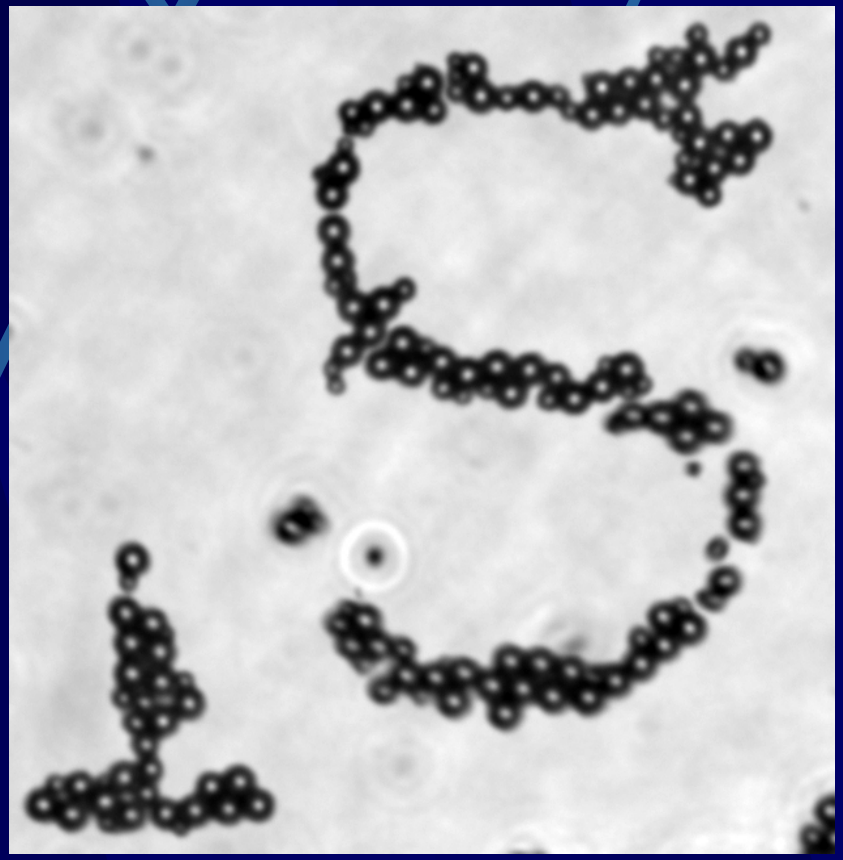


Figure by Tsinghua University, CLRF&CBM

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