

1

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## Design and organization of liver tissues

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2

### Typical flow in tissue engineering

3

### Hierarchical structure of the Liver

*The liver is the center of the metabolism!*

**Whole organ**

- 1,500 mL
- $2.5 \times 10^{11}$  cells

**Lobules**

- $\phi$  1-2 mm
- $5 \times 10^6$  cells

**How to make it in terms of per-volume-based functions?**

4

### Toward engineering implantable liver tissue

— 1/3 mass of human liver, 500 cm<sup>3</sup> —

**Preclinical research in pigs**  
= Establishment of the methodologies

- Growth of hepatocyte progenitors: → 3D culture
- Fabrication of 3D scaffolds: → design in terms of O<sub>2</sub> supply
- In vitro maturation to organ equivalents

● Implantation to pigs

● Acquisition of human hepatocytes  
• ES? MSC? iPS?

**Human clinical trials**

5

### Major approaches toward vascularized tissues

MEMS-related approach

3D fabrication-related approach

Modular assembly

Use of biological organization →

← Complete organization

6

### Practical approach

— balance between in vitro and in vivo organizations —

**Vacanti et al.**

“MEMS-based complete in vitro organization”

**Our practical approach**

**Sinusoidal-scale**

$\phi$  10  $\mu$ m

**Macro-scale**

1,500 cm<sup>3</sup>

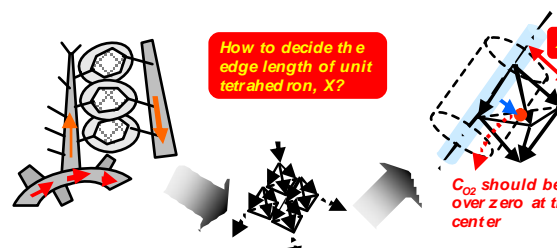
Expect... in vivo spontaneous organization upon implantation

In vitro artificial arrangement

● To what degree of microstructure we have to make in vitro?

### Concept for the scaffold design

- Minimally-necessary 3D branching/joining flow channel network (Macro-scale vasculature)
- Macroporous structure for cell growth around channels (We expect angiogenesis toward the center)



How to decide the edge length of unit tetrahedron, X?

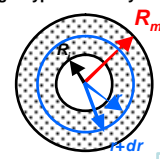
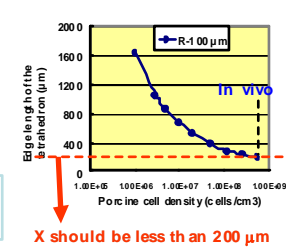
X

C<sub>O2</sub> should be over zero at the center

Sakai et al., *Mat. Sci. Eng. C*, 2004.

### How to decide the edge length of unit tetrahedron, X?

Krogh-type tissue cylinder

$\frac{d^2 C}{dr^2} + \frac{1}{r} \frac{dC}{dr} - \frac{\rho \cdot OCR}{D} = 0$   
 $C(r=R_c) = C_0$   
 $\frac{dC}{dr} \Big|_{r=R_c} = 0$   
 $C = C_0 + \frac{\rho \cdot OCR}{2D} \left[ \frac{r^2 - R_c^2}{2} + R_c^2 \ln \frac{R}{r} \right]$   
 $R_{max} = r \text{ when } C = 0$

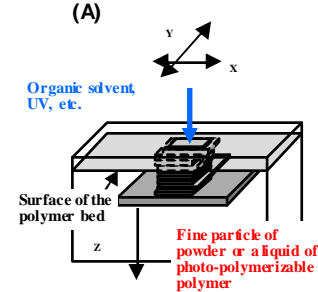
• ρ, Cell density, OCR, oxygen consumption rate  
 • D, diffusion

X should be less than 200 μm

(Krogh A., *J Physiol.*, 1919)

### Principle of 3D fabrication

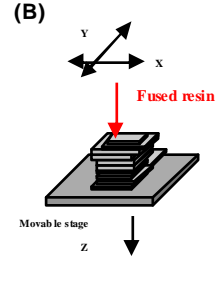
(A)



Organic solvent, UV, etc.

Fine particle of powder or a liquid of photo-polymerizable polymer

(B)

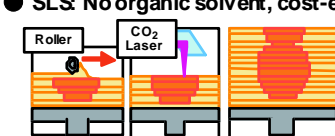


Fused resin

Surface of the polymer bed, Movab le stage, Z


### Fabrication of a 13 mL scaffold by the selective laser sintering (SLS) process

- SLS: No organic solvent, cost-effective...




- PCL Φ = 30 μm
- NaCl Φ = 100-200 μm (for macroporous structure)
- One layer, 200 μm
- Laser Φ = 0.5 mm

- Lower resolution than ideal!

**Ideal design**  


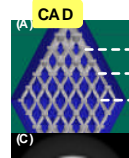
**Pre-manufacturing**  
 • Edge length of unit tetrahedron X = 4 mm  
 • Min Φ of slanting channels = 800 μm

**Practical design for manufacturing**  


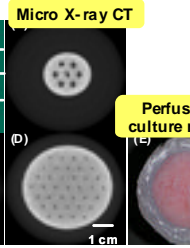
### Evaluation of the 3D scaffolds prepared

- SLS+ NaCl elution to generate macroporous structure

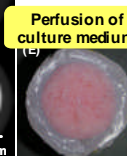
**CAD**



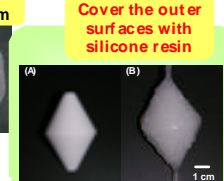
**Micro X-ray CT**



**Perfusion of culture medium**



**Cover the outer surfaces with silicone resin**



- Porosity = 89%
- Pore size Φ = 100-200 μm
- Min Φ of slanting channels = 800 μm

With Prof Nino and Technical Support Center at IS, Huang et al., *Biomater.*, 2006.

### Hep G2 cell inoculation and perfusion culture

- Avidin-biotin binding-based cell inoculation

**Avidin (MW = 68,000) + collagen for the scaffolds**


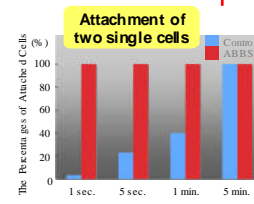
**Biotin for the cell surfaces**

- Covalent binding using Sulfo-NHS-LC Biotin (MW: 556.59)

**A-B complex**  
K<sub>d</sub> = 10<sup>-15</sup> M

Integrin-Laminin K<sub>d</sub> = 10<sup>-9</sup> M  
 Integrin-Fibronectin K<sub>d</sub> = 10<sup>-6</sup> M  
 Ab-Ag, K<sub>d</sub> = 10<sup>-9</sup> M

**Attachment of two single cells**

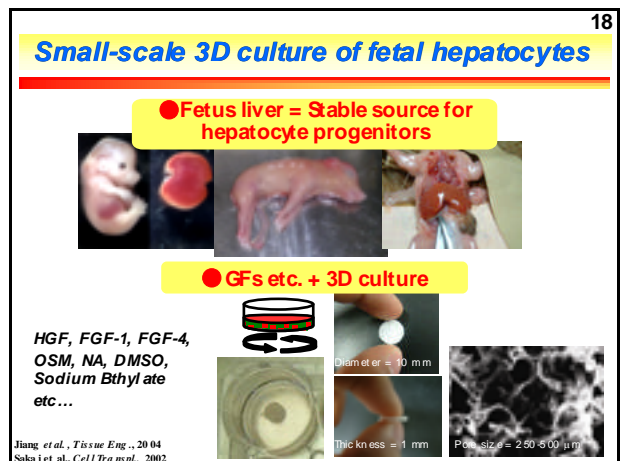
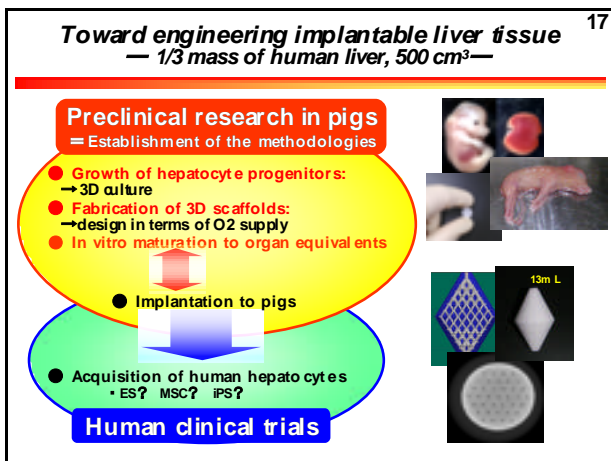
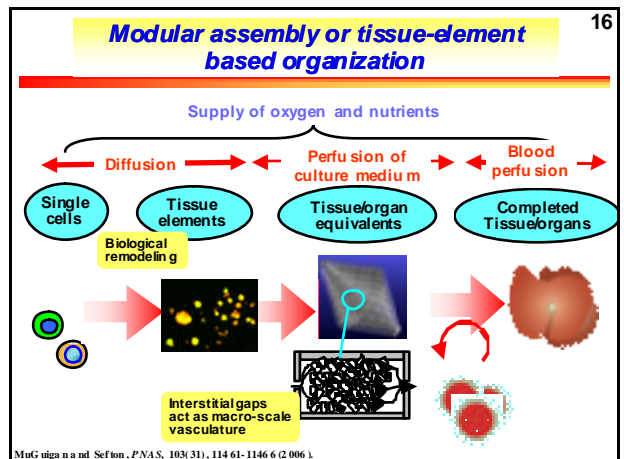
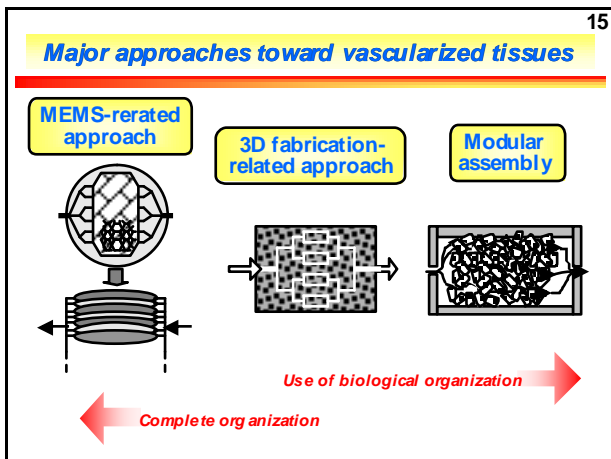
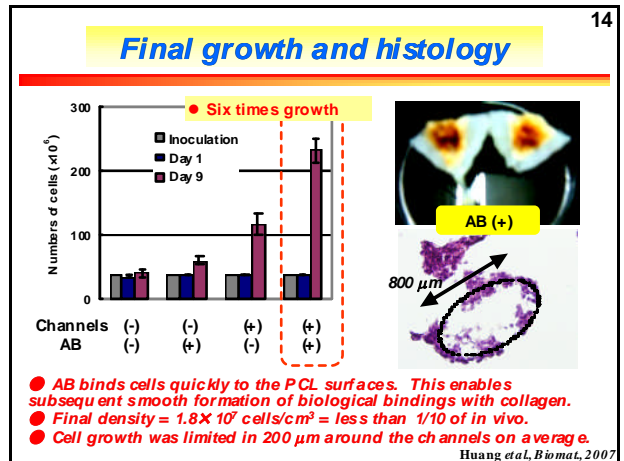
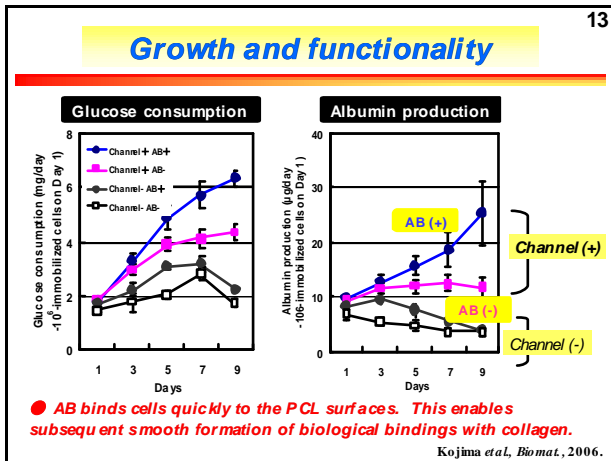



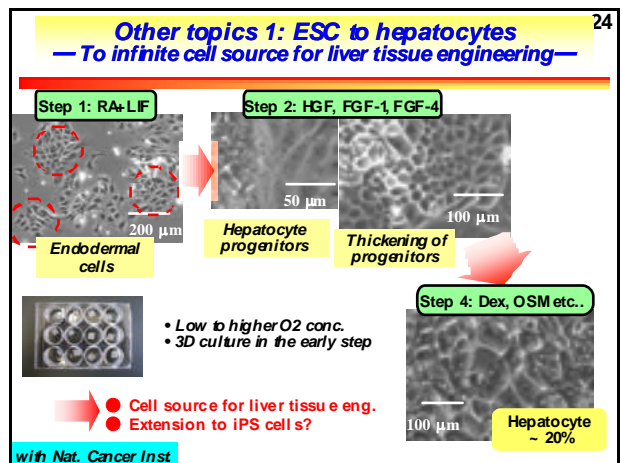
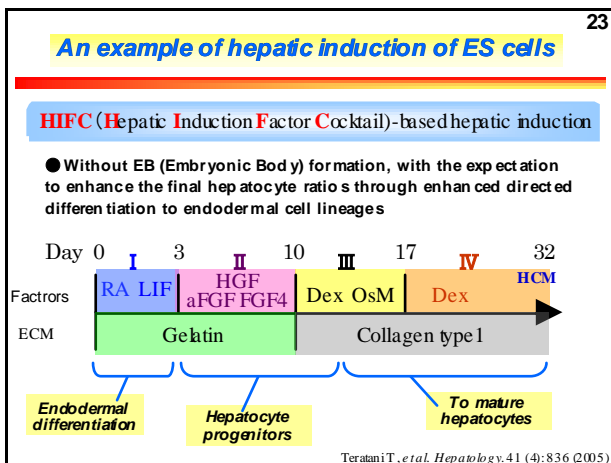
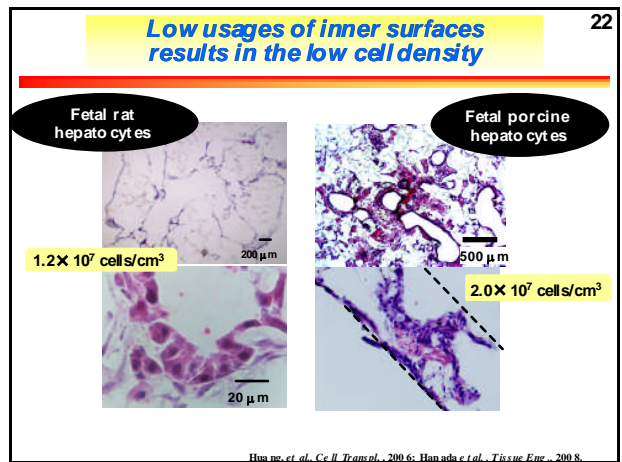
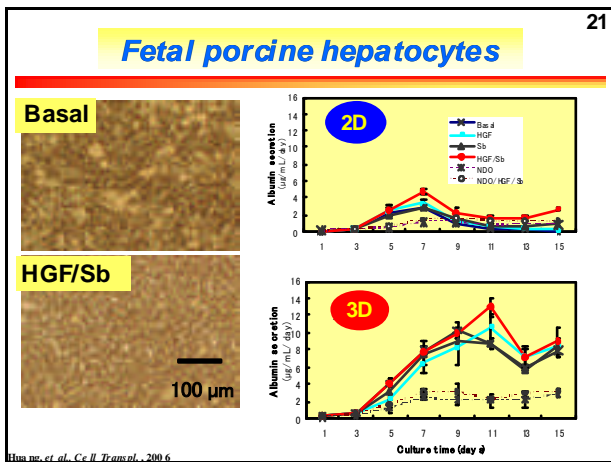
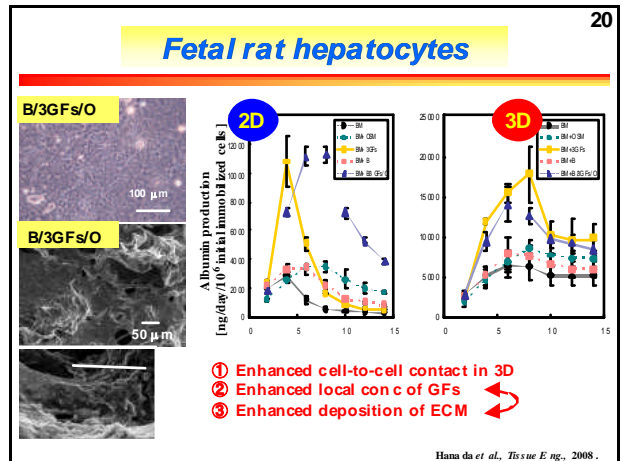
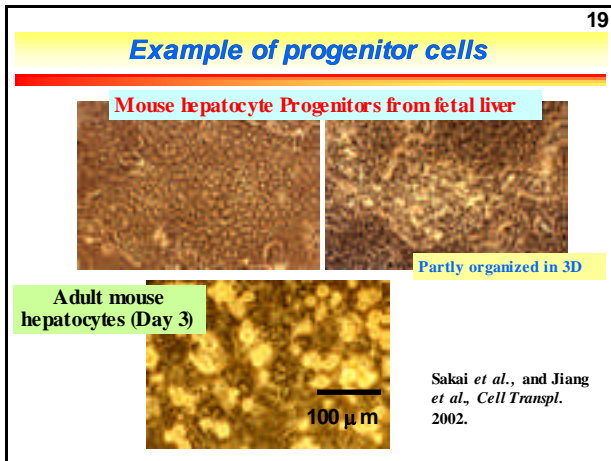
The Percentage of Attached Cells (%)

Control ABBS

Holding Time

Kojima et al., *Biomater.*, 2006.





**Toward engineering implantable liver tissue** — 1/3 mass of human liver, 500 cm<sup>3</sup>— 25

**Preclinical research in pigs**  
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**Human clinical trials**

**Necessity of hemoglobin-based oxygenation** — A fundamental dissolution of oxygen shortage — 26

- Low oxygen solubility decided by the Henry's law
- Flow rate limitation by the max. physiological shear stress (~10 dyn/cm<sup>2</sup>)

● 21%-O<sub>2</sub> can raise only up to 30 cm<sup>3</sup> liver tissue

● Feasibility of Hb-based O<sub>2</sub> carriers  
→ Toxicity? → Efficacy?

● PEGylated liposome-encapsulated hemoglobin (Oxygenix Co.Ltd.)

**Efficacy of LEH in adult rat hepatocytes** 27

**2D flat-plate bioreactor**

O<sub>2</sub> saturated O<sub>2</sub> depleted

● 20% LEH, 3-days exposure

LEH (-) LEH (+)

**Albumin production**

Days in culture	Static LEH	Perfusion LEH
1	~15	~15
2	~18	~45
3	~12	~25
4	~10	~22

Albumin production (μg/day/10<sup>6</sup> cell)

Narita, H. et al., *J. Biosci. Bioeng.* 2007.

**Toxicity of LEH in fetal rat hepatocytes** — Differences in cellular uptake? — 28

- 20% LEH, Fetal hepatocytes, 14-days exposure
- Cellular uptake

Completely avoid the cellular intake!

- ▼ Increase the diameter?
- ▼ Improve the surface modification?

● Intracellular radical formation?  
● Low ability to scavenge radicals in fetal hepatocytes?

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**Human clinical trials**

- 3D fabrication having higher resolution
- Low toxicity O<sub>2</sub> carriers
- Endothelialization of the channel
- Angiogenesis from the channel
- Bile duct network over the scaffold
- in vivo evaluation
- Cost-effective protocol for differentiation