# HST 535
# Principles and Practice of Tissue Engineering

## Effects of Culture Conditions

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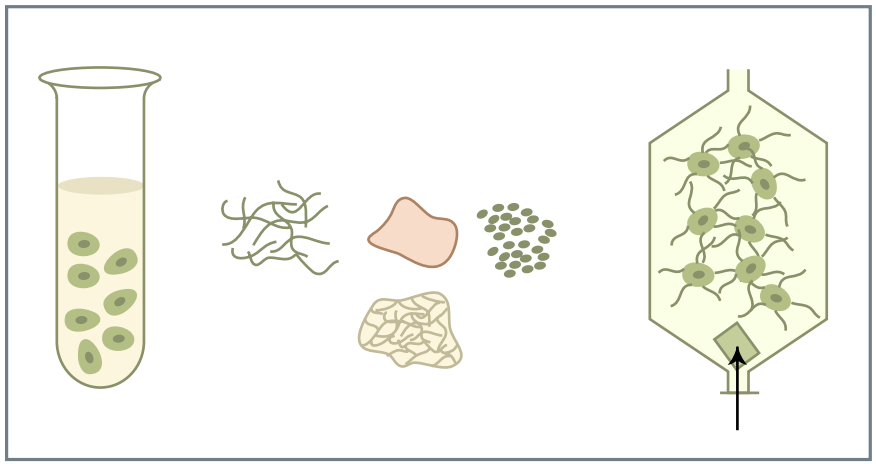
# Tissue engineering approach



Cells + Biomaterial + Bioreactor

Engineered Tissue

Transplantation

Figure by MIT OCW. After Vacanti and Langer.

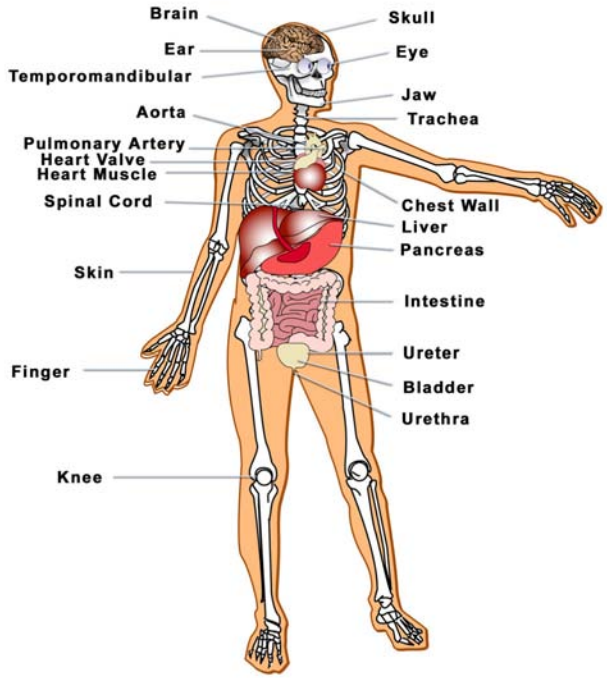



Figure by MIT OCW.

adapted from Vacanti & Langer, *Lancet* 354:32, 1999

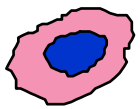
# Culture conditions determine cell fate

Physiological milieu (temperature , pH, oxygen)
3-dimensional environment, cell-to-cell contact)

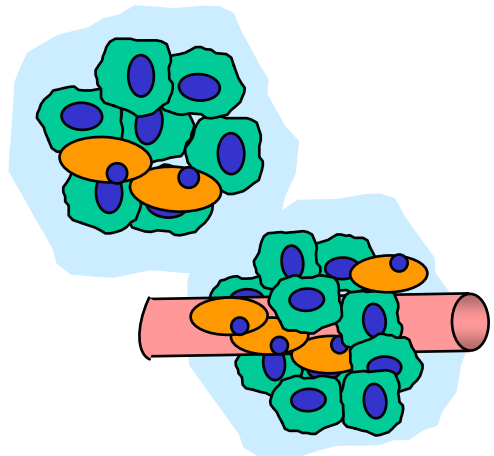
Biochemical factors (nutrients, growth factors)

Physical stimulation (compression, tension)

Cell



Differentiated Tissues



Loss of specialized function
senescence (aging), death

# Culture conditions key for tissue engineering

1. ***Defined Culture Media***, **to induce differentiation of progenitor (stem) cells.**

2. ***Biomaterial Scaffolds,*** **to provide a 3-D cell culture environment, and to influence the mechanical properties of engineered tissue.**

3. ***Bioreactor Vessels,*** **to promote spatially uniform cell seeding, and to provide mass transport and biophysical stimulation during tissue culture.**

# Defined media → cell differentiation

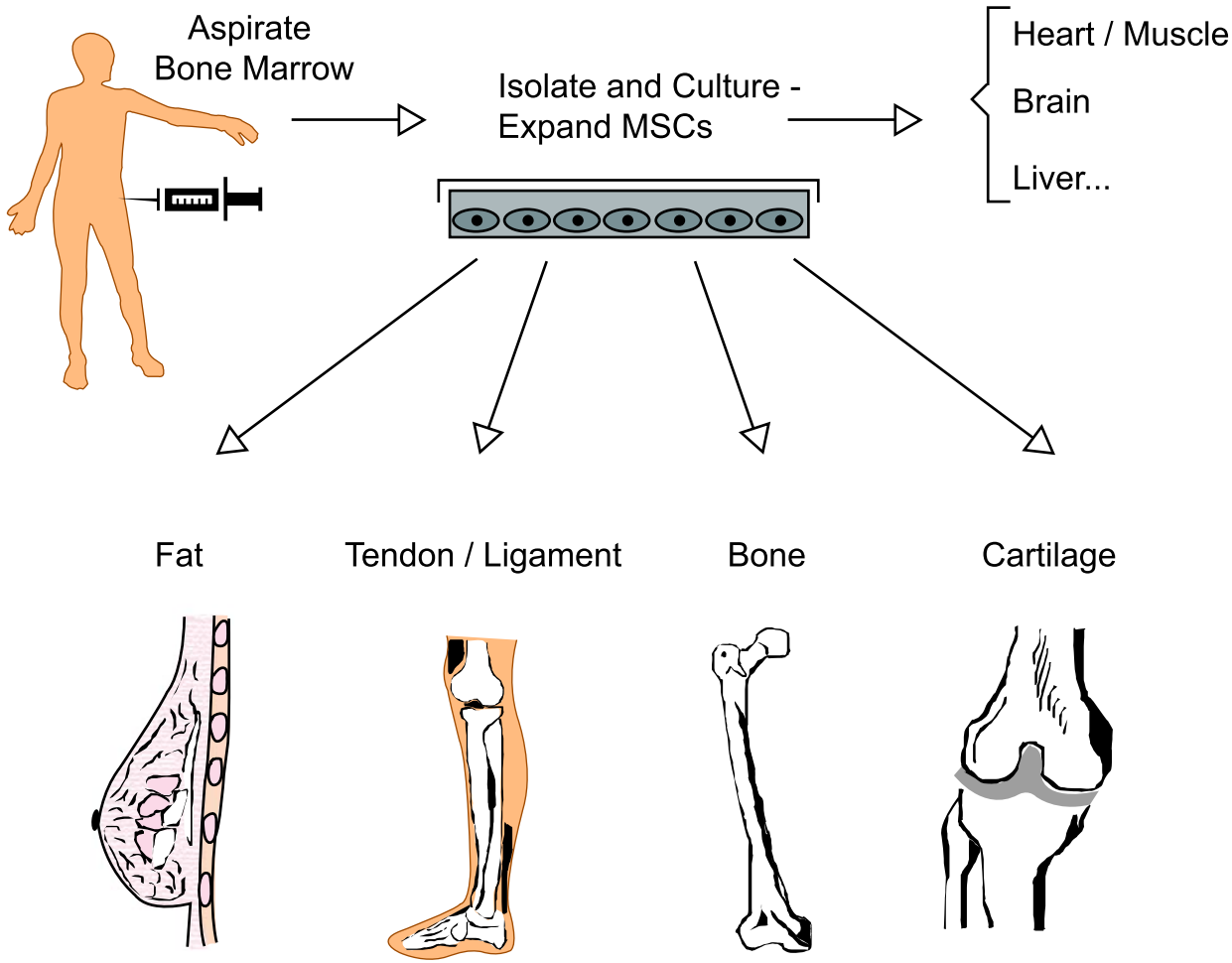
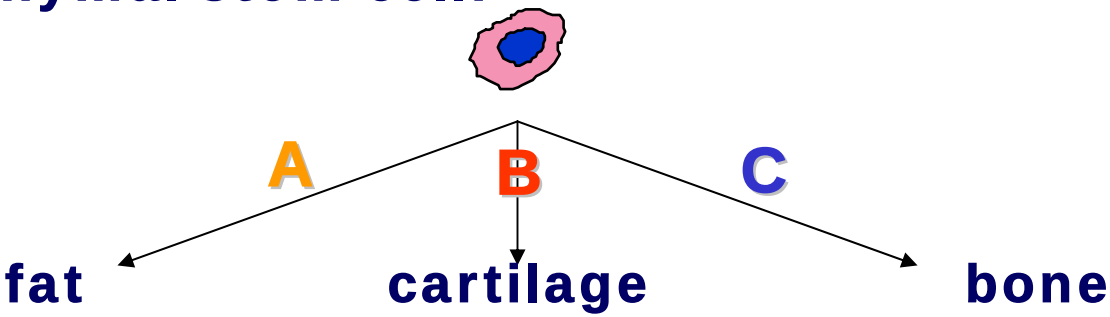



Figure by MIT OCW. After Bruder & Caplan, *Principles of Tissue Engineering*, 2000.

# Medium A,B, or C→ selective differentiation

**mesenchymal stem cell:**




donor #1

Grid of six photos removed for copyright reasons.

donor #2

Lipid Type II collagen Alkaline phosphatase

Pittenger et al., *Science* 284: 143, 1999

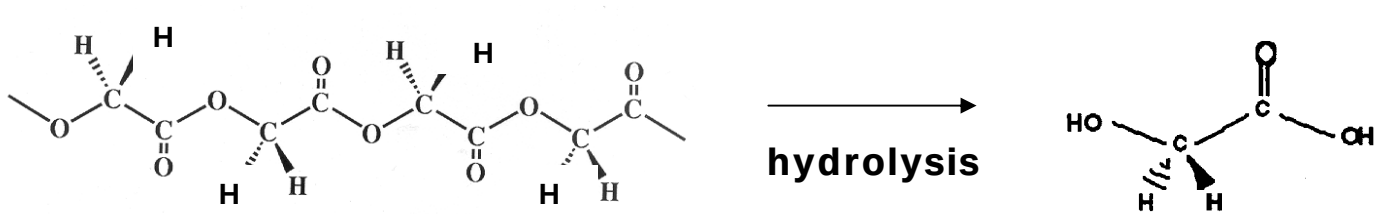
# A scaffold → 3-dimensional culture
## (non woven mesh of polyglycolic acid, PGA)

Two photos removed for copyright reasons.

2 mm

20 µm

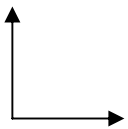
**Fiber diameter (13 µm) is similar to that of a cell;**
**Porosity is high (97 %); material is biocompatible.**




Source: Freed, L., et al. (Nat)Bio/Technol 12: 689, 1994.

# 3-D scaffold promoted differentiation

(chondrocyte cultures)



**PGA fiber**

Image removed for copyright reasons.

Source: Freed, L., et al. (Nat)Bio/Technol 12: 689, 1994.

# 3-D scaffolds for engineering cartilage

(representative)

**Three photos** removed for copyright reasons.

| 3-D structure | sponge | non-woven mesh | woven mesh |
|---|---|---|---|
| structural stability | higher | lower | intermediate |
| porosity, pore inter-connectivity | lower | higher | intermediate |

# Appropriate biomechanics with meshes

(chondrocytes on different scaffolds, 1 month)

**benzylated hyaluronan**

**sponge** **NW mesh**

**polyglycolic acid**

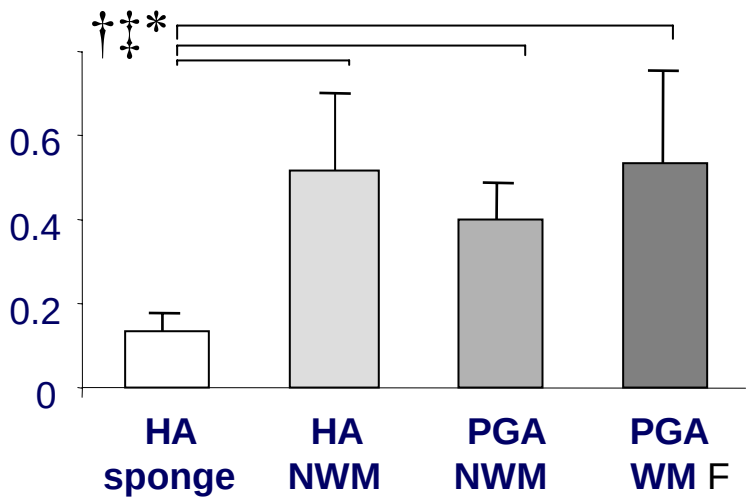
**NW mesh** **woven mesh**

**Scaffold:**

Four photos removed for copyright reasons.

50 μm 500 μm

**Modulus (MPa) constructs cultured for 4 weeks**

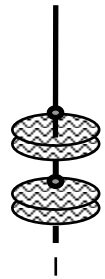



Pei et al., *FASEB J.* 16: 1691, 2002

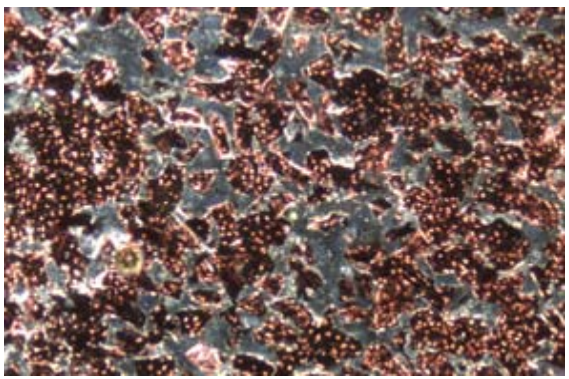
# 3-D cell seeding requires dynamic mixing

(chondrocytes on sponge or mesh)

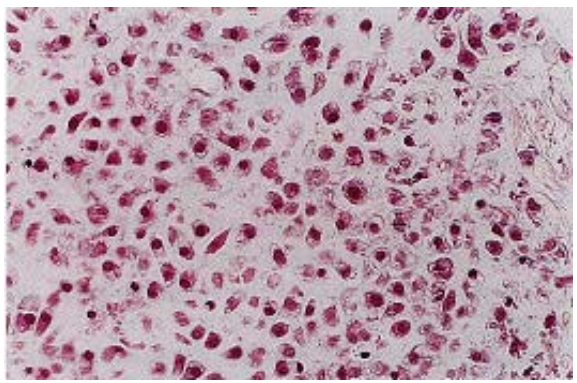
Two photos removed for copyright reasons.



**Porous sponge:**



**Fibrous mesh:**



Photos by L. Freed. See Vunjak-Novakovic, G. et al. *Biotechnol Prog*. 14 no. 2 (1998): 193-202

# 3-D scaffolds for engineering heart

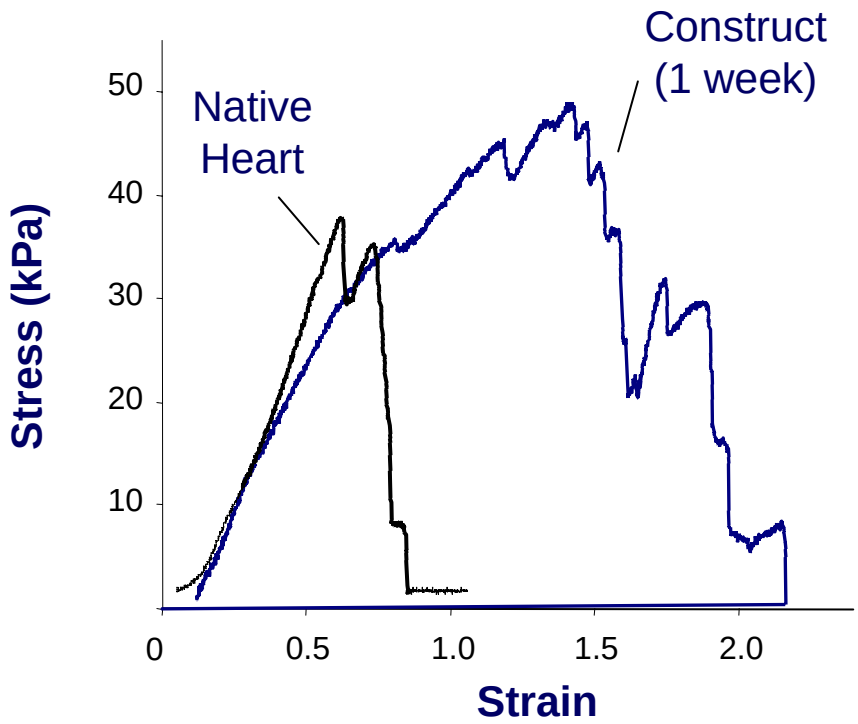
(representative)

Three photos removed for copyright reasons.

| | | | |
|---|---|---|---|
| **3-D structure** | **non-woven mesh** | **sponge** | **knitted fabric** |
| **Structural stability** | **low** | **low** | **high** |
| **Porosity, pore inter-connectivity** | **high** | **high** | **non-uniform** |

# Appropriate biomechanics with knitted fabric

(heart cells/gel on knitted fabric, 1 week)




Boublik et al., Tissue Eng. 11: 1122, 2005.

# 3D cell seeding requires hydrogel & perfusion

(C2C12 myocytes/gel on collagen sponge, 5 h)

**petri dish** **perfusion**

**Top**

Image removed for copyright reasons.

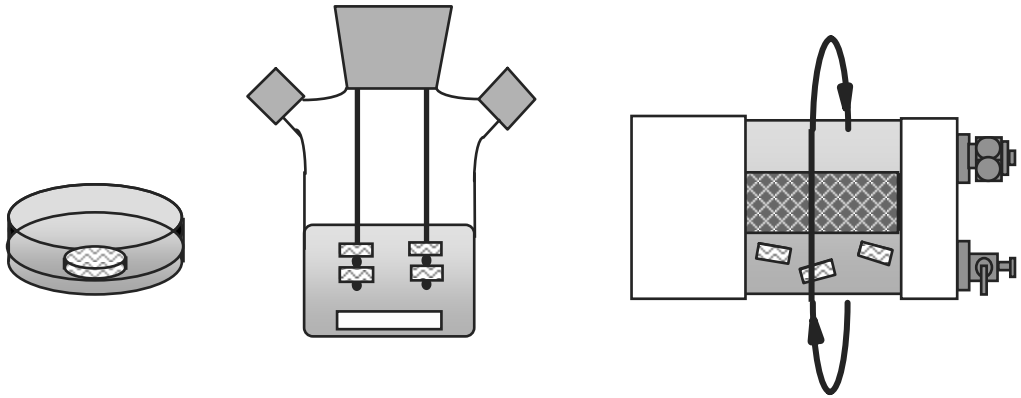
**Center**

**Bottom**

100 μm

*Radisic et al., Biotech Bioeng 82: 403, 2003*

# Culture systems for engineering cartilage



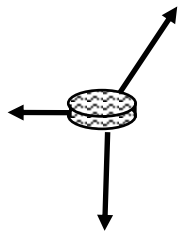
| | static petri dish | spinner flask | rotating bioreactor |
|---|---|---|---|
| Mechanism of mixing | none | magnetic stirring | rotational flow, construct settling |
| Flow pattern | none | turbulent | laminar |
| Gas exchange | surface aeration | surface aeration | internal membrane |
| Mass transfer | diffusion | convection | convection |

# Rotating bioreactor
## → dynamic, laminar flow pattern

**side view:**

**end view:**

**suspended construct:**



Images removed for copyright reasons.

Flow-visualization study and video by P. Neitzel

Freed & Vunjak-Novakovic, *Biotech Bioeng* 36: 306, 1995

# Chondrogenesis in rotating bioreactor

**culture day 12:** **culture day 40:**

**Glycosaminoglycans (safranin-O stain)**

2 mm

Image removed for copyright reasons.

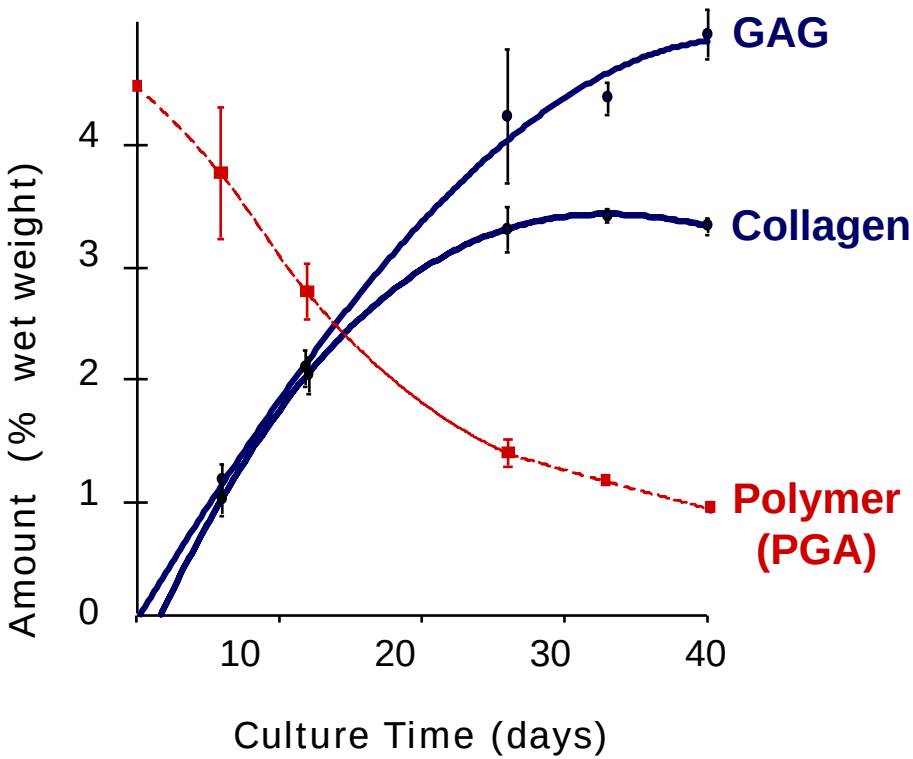
**Type II Collagen (immunostain)**

Freed et al., *Exp Cell Res* 240: 58, 1998

# Bioreactor vs. conventional culture

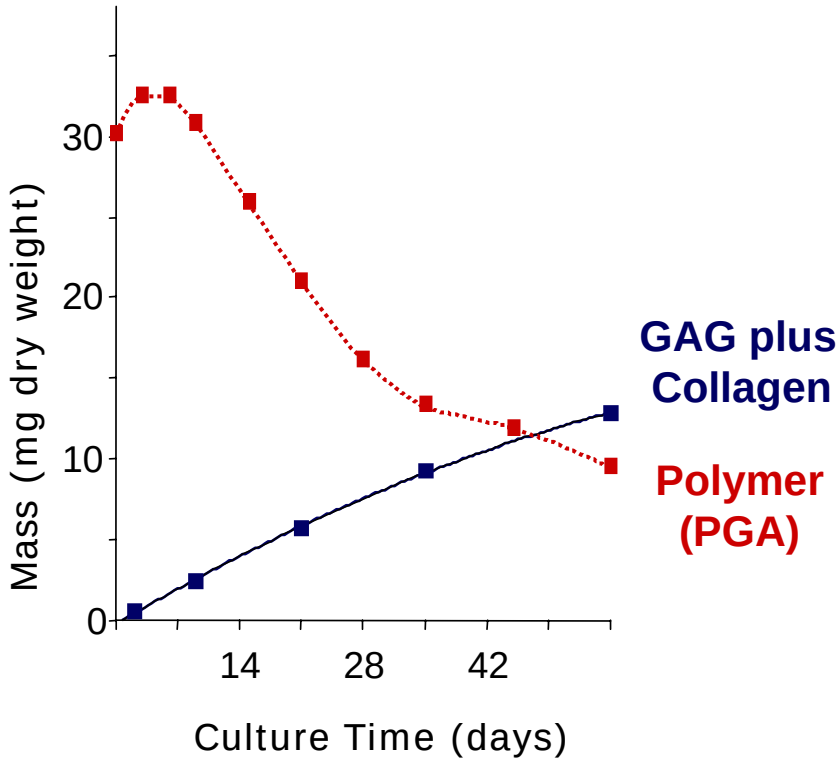
(for chondrogenesis)

## Rotating bioreactor:




*Exp Cell Res* 240:58, 1998

## Petri dish:




*Bio/Technol* 12:689, 1994

# Bioreactors improve size and structure

(engineered cartilage)

**benzylated hyaluronan**

**sponge** **NW mesh**

**polyglycolic acid**

**NW mesh** **composite**

**Bioreactor:**

Image removed for copyright reasons.

**Petri dish:**

1 mm

Pei et al., *FASEB J.* 16: 1691, 2002

# Bioreactors improve molecular properties

(engineered cartilage)

**Bioreactor:** **Petri dish:**

**Type I Collagen (Western blot)**

**Type II Collagen (Western blot)**

Image removed for copyright reasons.

Figure 6 from Pei et al 2002.

$\alpha 1/2(I)$

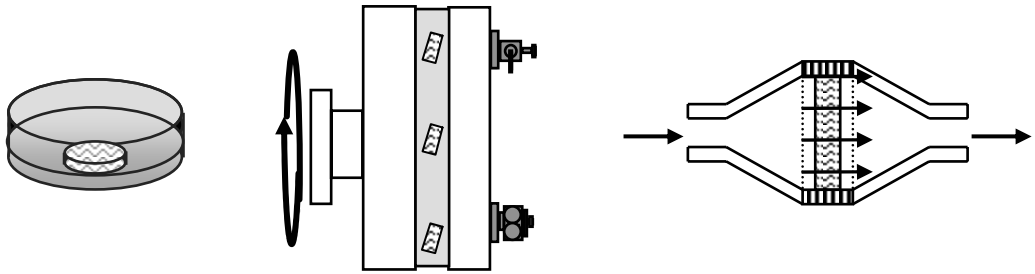
$\alpha 1(II)$

**Type I Collagen (band intensity)**

**Type II Collagen (band intensity)**

Pei et al., *FASEB J.* 16: 1691, 2002

# Culture systems for engineering heart



| | static petri dish | rotating bioreactor | perfused cartridge |
|---|---|---|---|
| Mechanism of mixing | none | rotational flow, construct settling | recirculation of medium |
| Flow pattern | none | laminar | laminar |
| Gas exchange | surface aeration | internal membrane | external membrane |
| Mass transfer | diffusion | convection | convection |

# Controlled *in vitro* studies

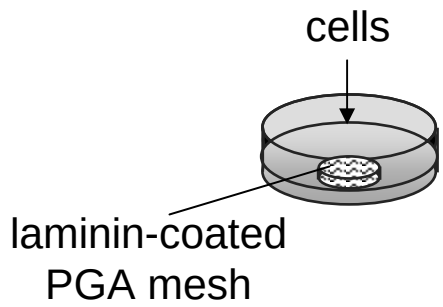
(3-D construct vs. conventional culture conditions)

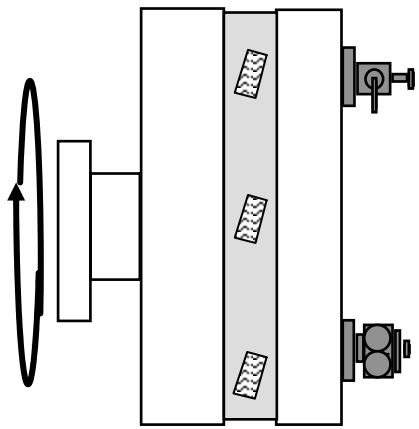
**3-D construct:**

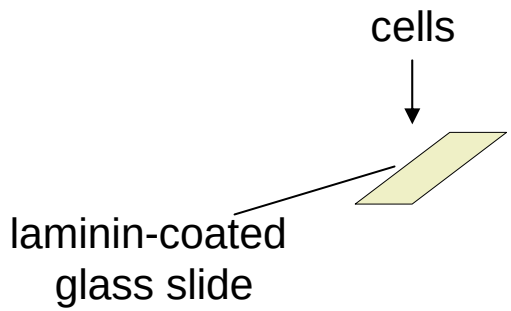
**monolayer:**

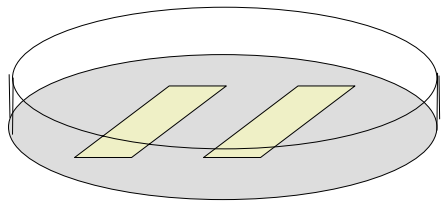
**seeding**

**cultivation**



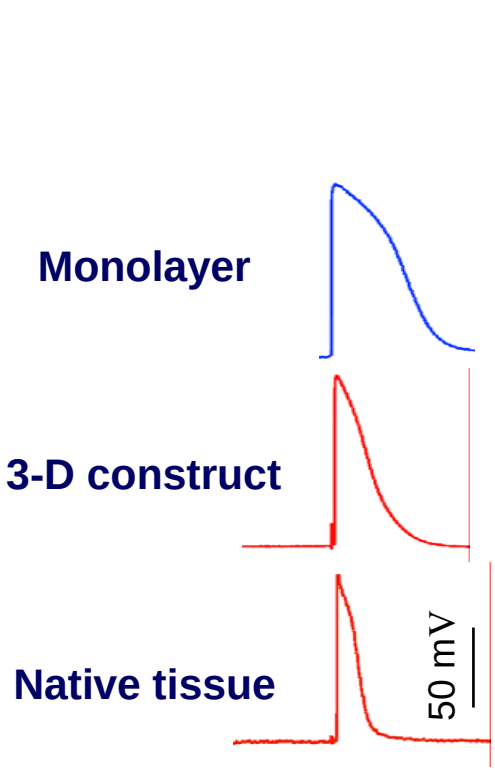



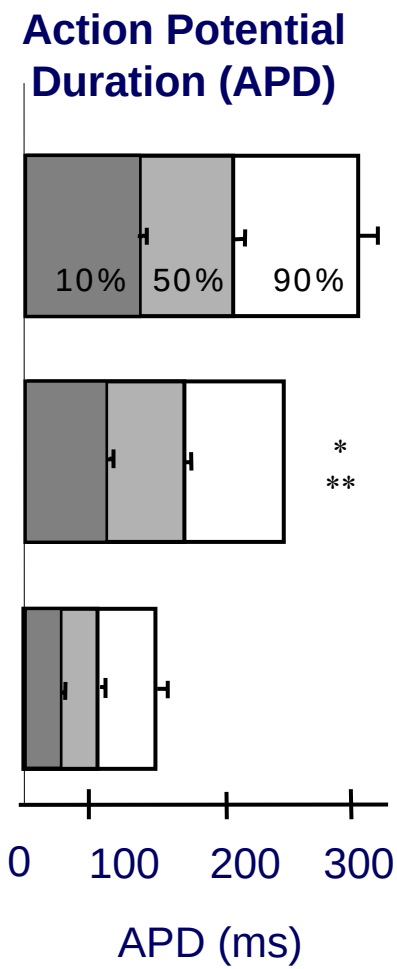


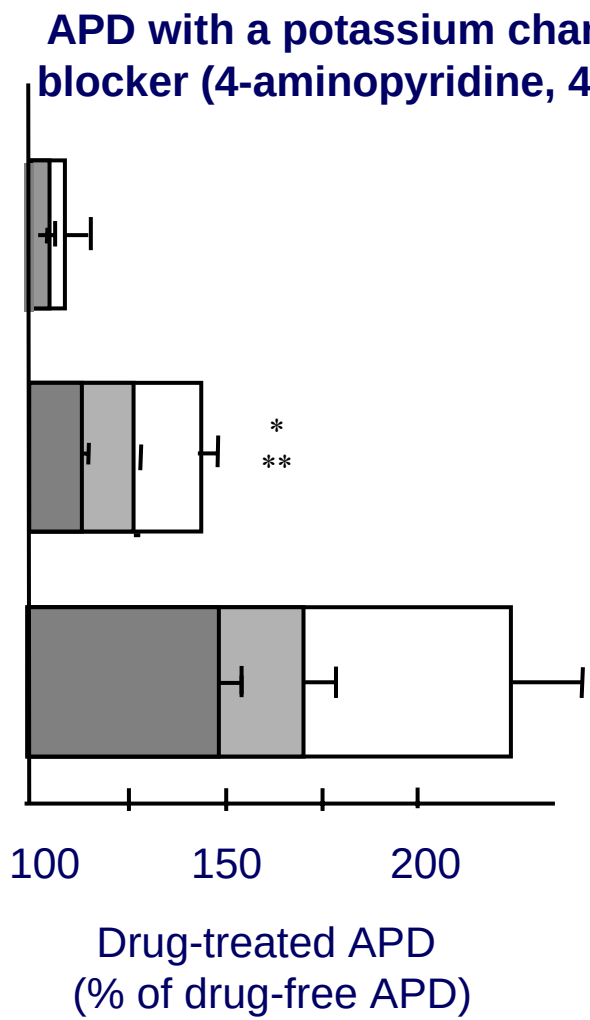



Bursac et al., *Tissue Eng.* 9: 1243, 2003

# Appropriate electrical properties with heart cells cultured on scaffold in bioreactor




Bursac et al., *Tissue Eng.* 9: 1243, 2003

# Summary

**Culture conditions key for tissue engineering:**

- ***Defined culture media*** **induce cell differentiation by providing key regulatory factors**
- ***Biomaterial scaffolds*** **further enhance cell differentiation by providing a 3-D culture environment, and can influence mechanical properties of engineered tissues.**
- ***Bioreactors*** **improve cell seeding and functional tissue development by providing mixing, mass transport, and biophysical stimulation.**

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