Harvard-MIT Division of Health Sciences and Technology
HST.535: Principles and Practice of Tissue Engineering
Instructor: Shuguang Zhang

# Fabricate Biological Nanomaterials

# For Tissue Engineering

## HST-535

## Wednesday, 17 Sept., 2003

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http://web.mit.edu/lms/www

# ***Tissue Engineering requires 2 key ingredient:***

- ***Stem cells***
  - ***Embryonic stem cells***
  - ***Adult stem cells***
- ***Biological scaffolds***
  - ***Polymer inert scaffolds***
  - ***Designed functional biological scaffolds***

# *Self-assembly is ubiquitous in Nature*

## Each fish is about 5--50 centimeters in length

Photo courtesy of USGS.

# *Molecular Self-assembly of Tetrameric Hemoglobin Polypeptides*

Image removed for copyright reasons.

**6.4 nm in diameter**

http://web.mit.edu/lms/www

# ***Molecular Self-assembly Through Weak Interactions***

- **Hydrogen Bonds**

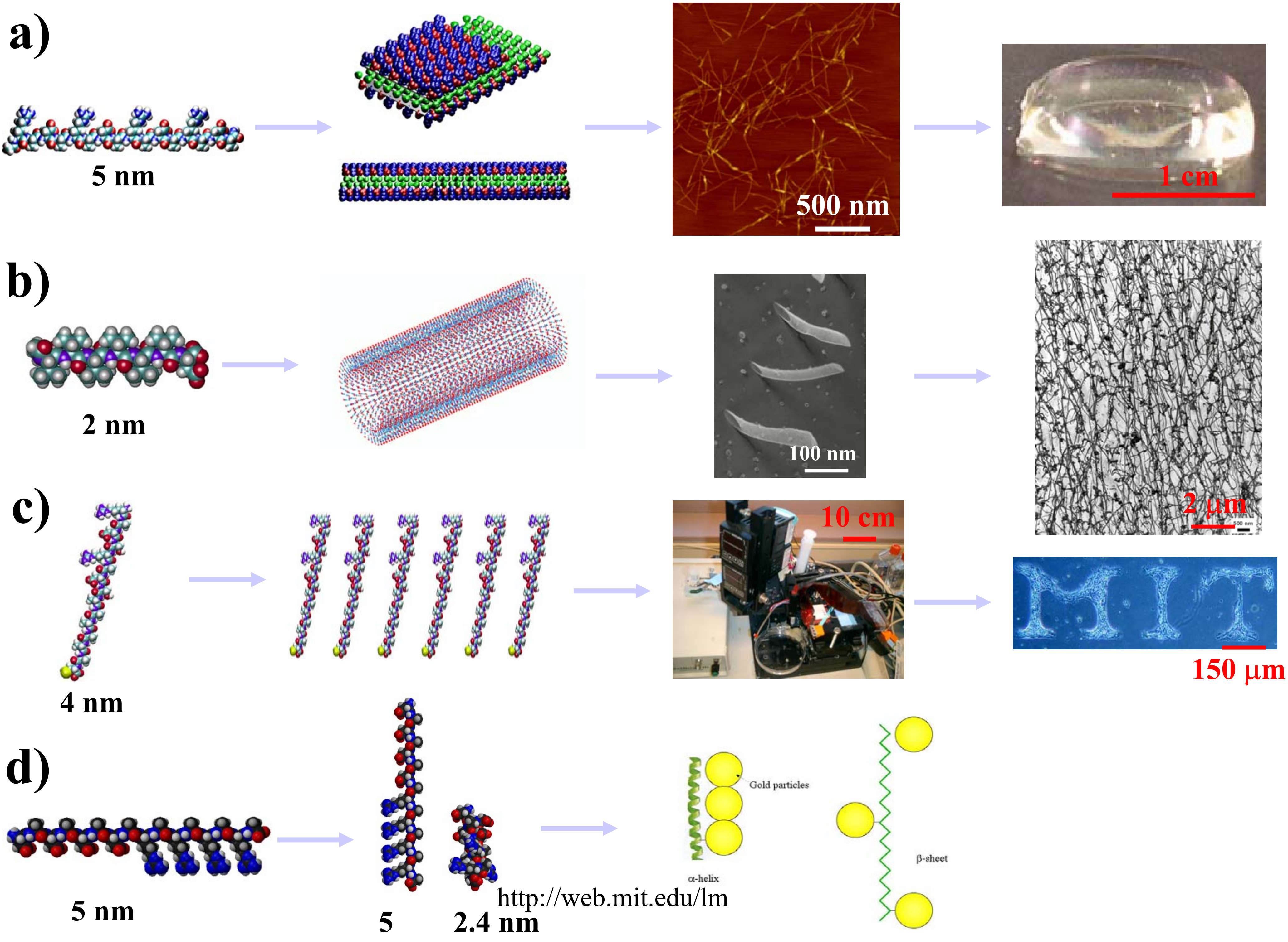
- **Ionic Bonds (Electric static interaction, salt bridges)**

- **van der Waals Interactions**

- **Hydrophobic interactions**

- **$H_2O$ mediated interactions (Water-mediated H-bonds)**

http://web.mit.edu/lms/www


a)
5 nm
500 nm
1 cm
b)
2 nm
100 nm
2 μm
c)
10 cm
4 nm
150 μm
d)
5 nm
Gold particles
α-helix
β-sheet
http://web.mit.edu/lm
5
2.4 nm

# <u>Introducing:</u>

**the self-assembling peptide nanofiber scaffold to culture, to grow and to study tissue cells in 3-dimensions:**

***<u>"Molecular Cement"</u>***

http://web.mit.edu/lms/www

# ***Discovery of Peptide Nanofibers Scaffolds***

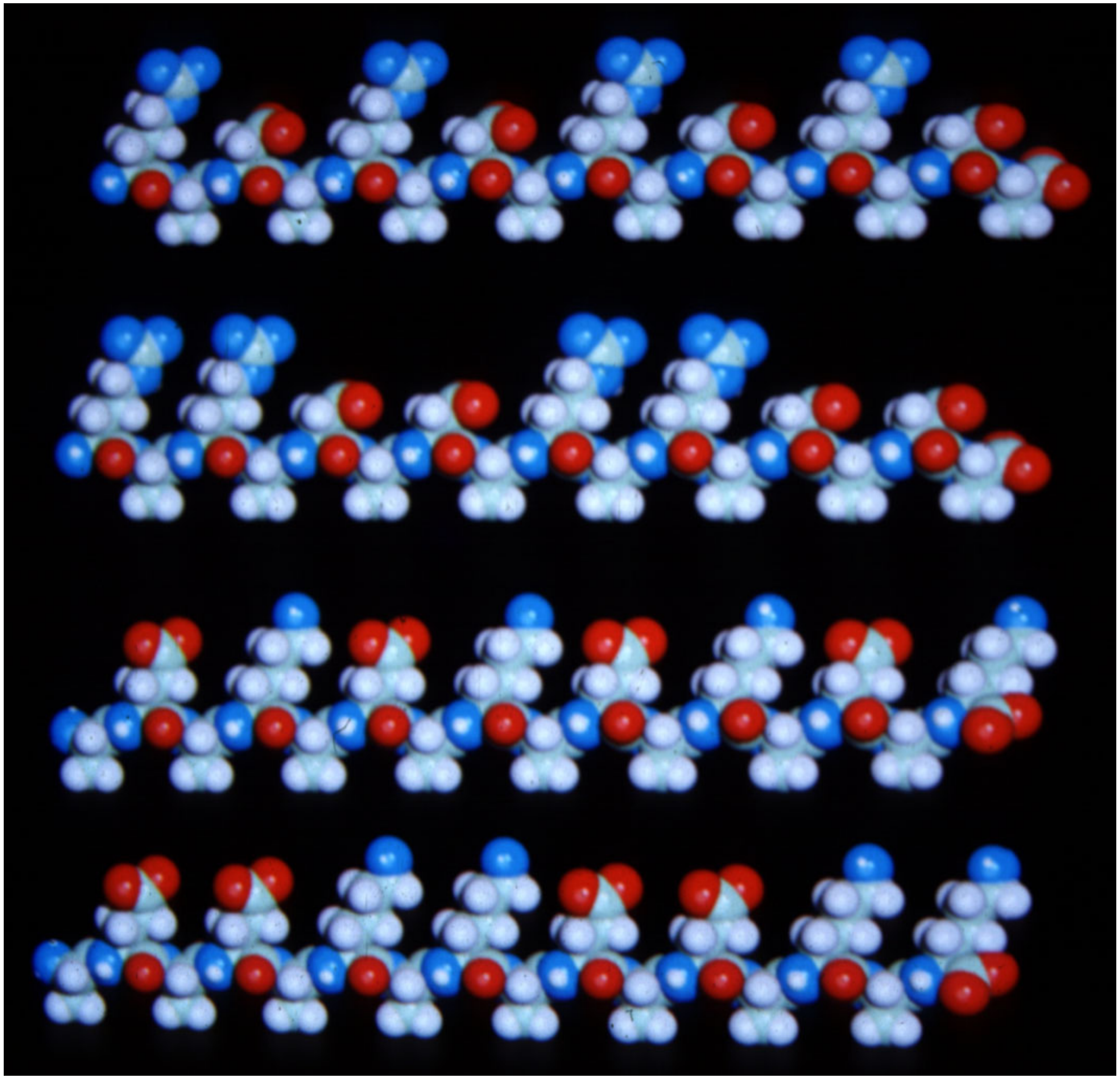
# ***Self-assembling Peptides Inspired from Nature***

**RAD16-I**

**RAD16-II**

**EAK16-I**

**EAK16-II**



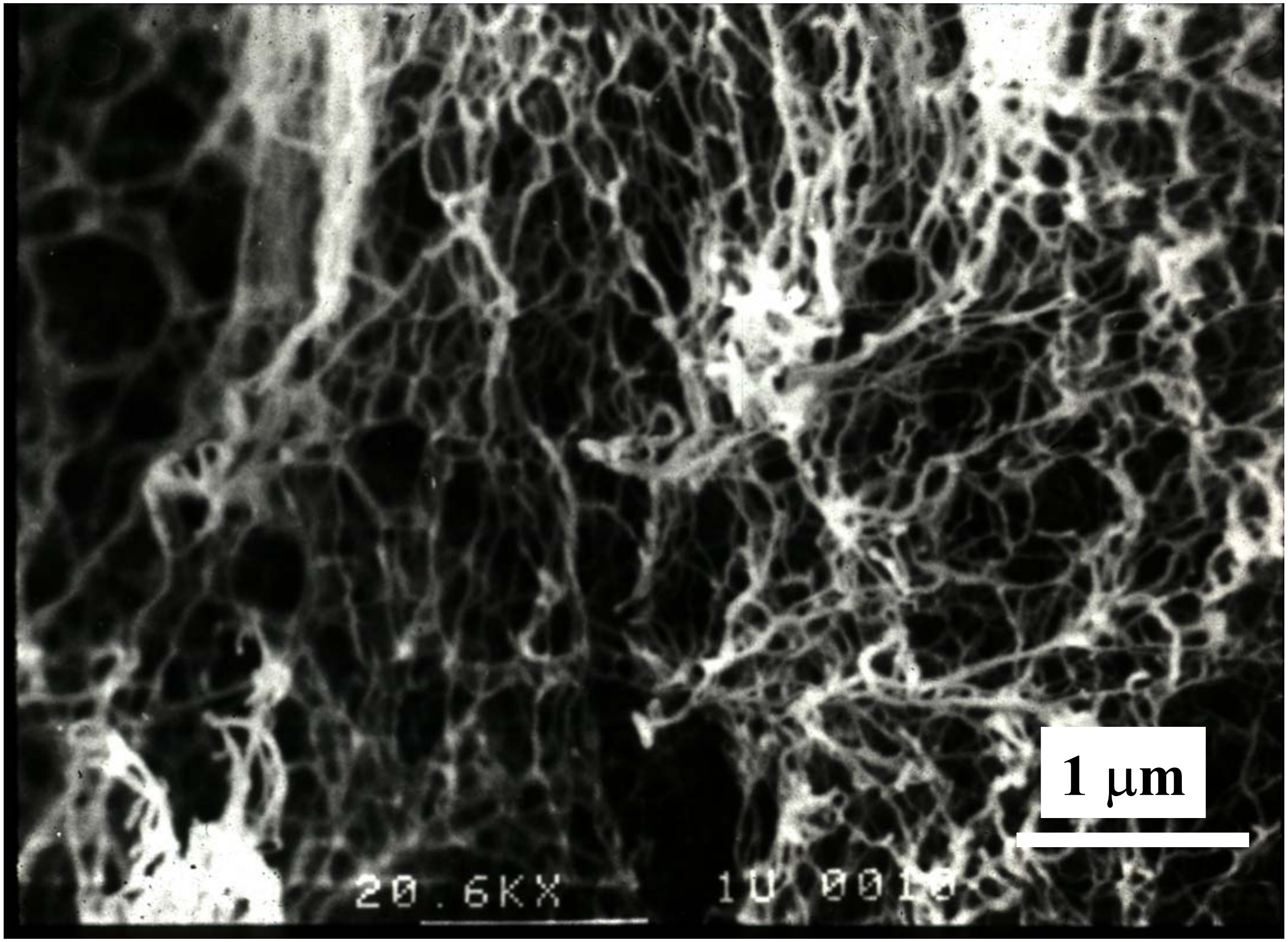
**Found in a yeast protein, Zuotin**

**Zhang, et al., *PNAS*, April, 1993, Zhang, et al., *Biomaterials*, Dec. 1995**

http://web.mit.edu/lms/www

# Self-assembling Peptide Nanofibers

## Scanning EM Image, EKA16-II

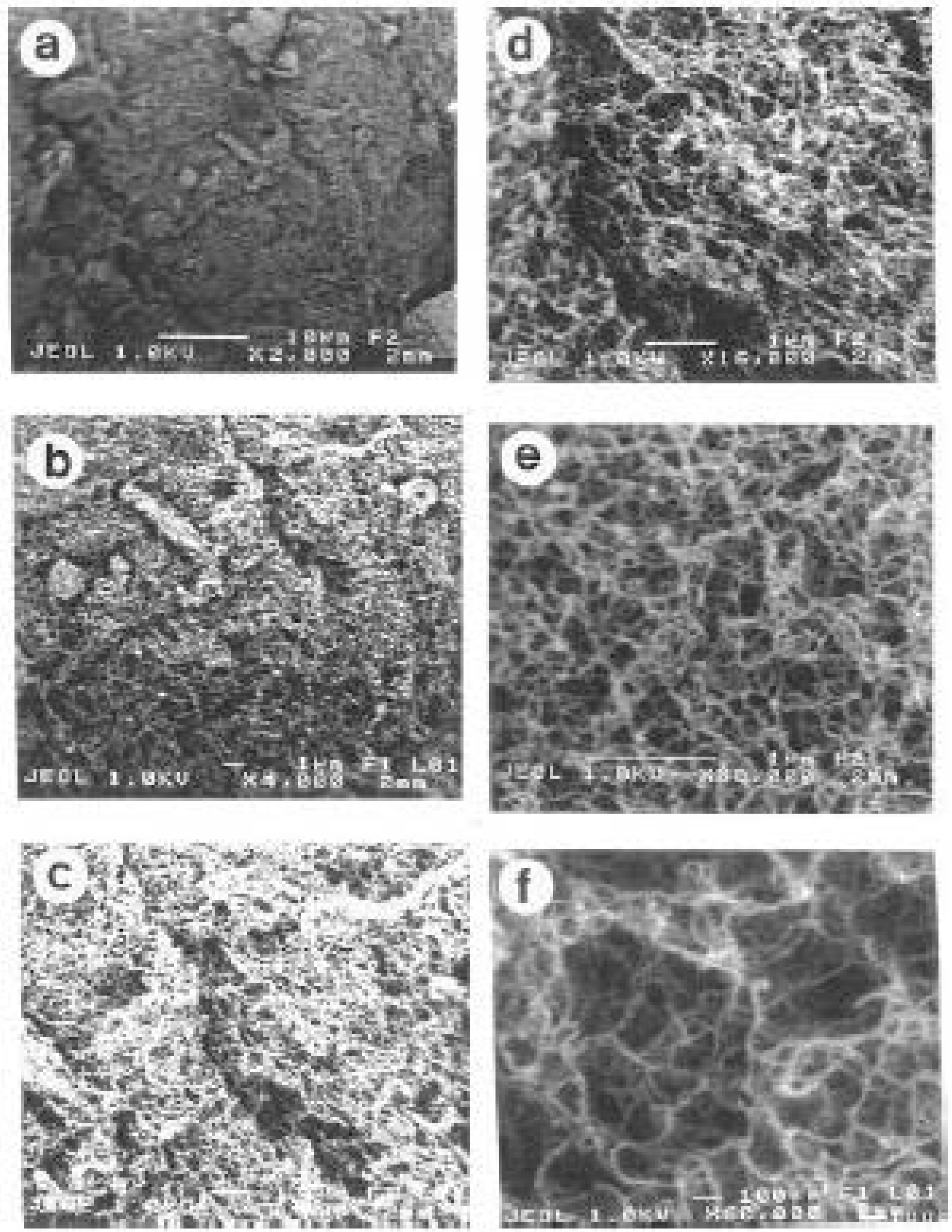



**Zhang, et al., *PNAS*, April, 1993** http://web.mit.edu/lms/www

# Self-assembling peptide nanofiber scaffold

**RADA16-I**

**(SEM)**



http://web.mit.edu/lms/www

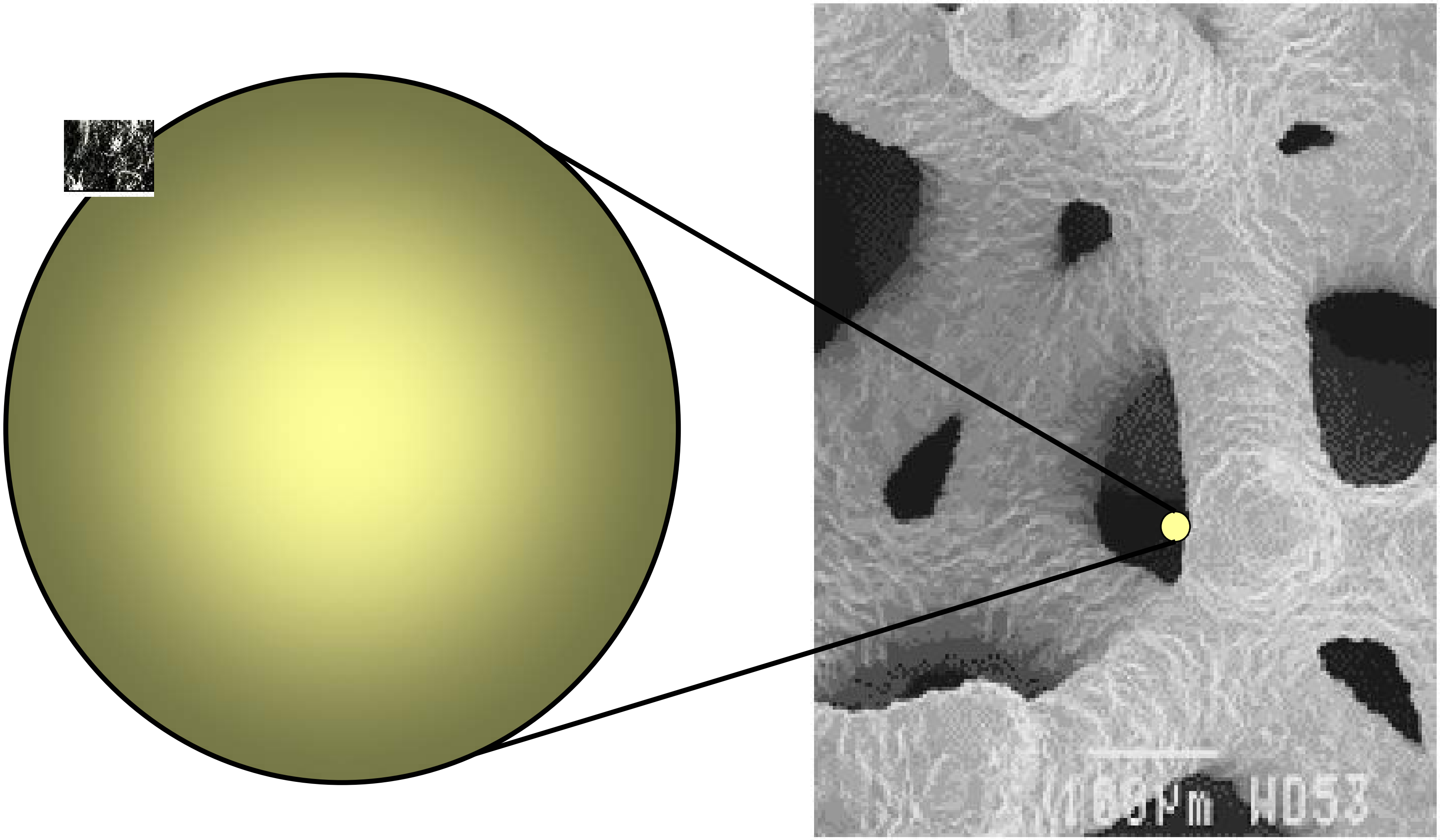
# ***Biopolymers PGA/PLLA microfibers used as scaffold***

Photo removed for copyright reasons.

**Mikos, et al., (1993) *J. Biomed. Mater. Res*. 27, 183-189**
**The yellow dot is about the size of most tissue cells.**

http://web.mit.edu/lms/www

# Drastic Size Difference



**Typical cell dwarfs the peptide scaffold microstructure, with nanometer-sized fibers and pores which structure and hold large amounts of water and nutrients.**

**Typical synthetic matrix far too large and rigid for cells to truly grow in 3D.**

# ***Molecular Model of EFK8 (FEFKFEFK)***

**(Davide Marini, Mechanical Engineering, MIT)**

**0.9 nm**

**1.4 nm**

Molecular diagram removed
for copyright reasons.

**2.5 nm**

**Marini, et al., *NanoLetters*, April, 2002**

# ***Atomic force Microscopy Image of EFK8 (FEFKFEFK)***

**8 minutes after in water (Davide Marini, Mechanical Engineering, MIT)**

Figure removed for
copyright reasons.

**Marini, et al., *NanoLetters*, April, 2002**

# Atomic force Microscopy Image of EFK8

**2 hours after in water (Davide Marini, Mechanical Engineering, MIT)**

Figure removed for
copyright reasons.

**Marini, et al., *NanoLetters*, April, 2002**

# *AFM & Cryo-TEM images of EFK8 nanofiber intermediate*

## Davide Marini, Mechanical Engineering

### Marini, et al., *NanoLetters*, April, 2002

**AFM** **Cryo-TEM**

Two figures removed for copyright reasons.

**Left-handed double helix**

http://web.mit.edu/lms/www

# *The molecular modeling & simulations*

**Marini, et al., *NanoLetters*, April, 2002**

7 nm

Axis view

Diagrams removed for
copyright reasons.

19 nm

Inner sheet
(S13)

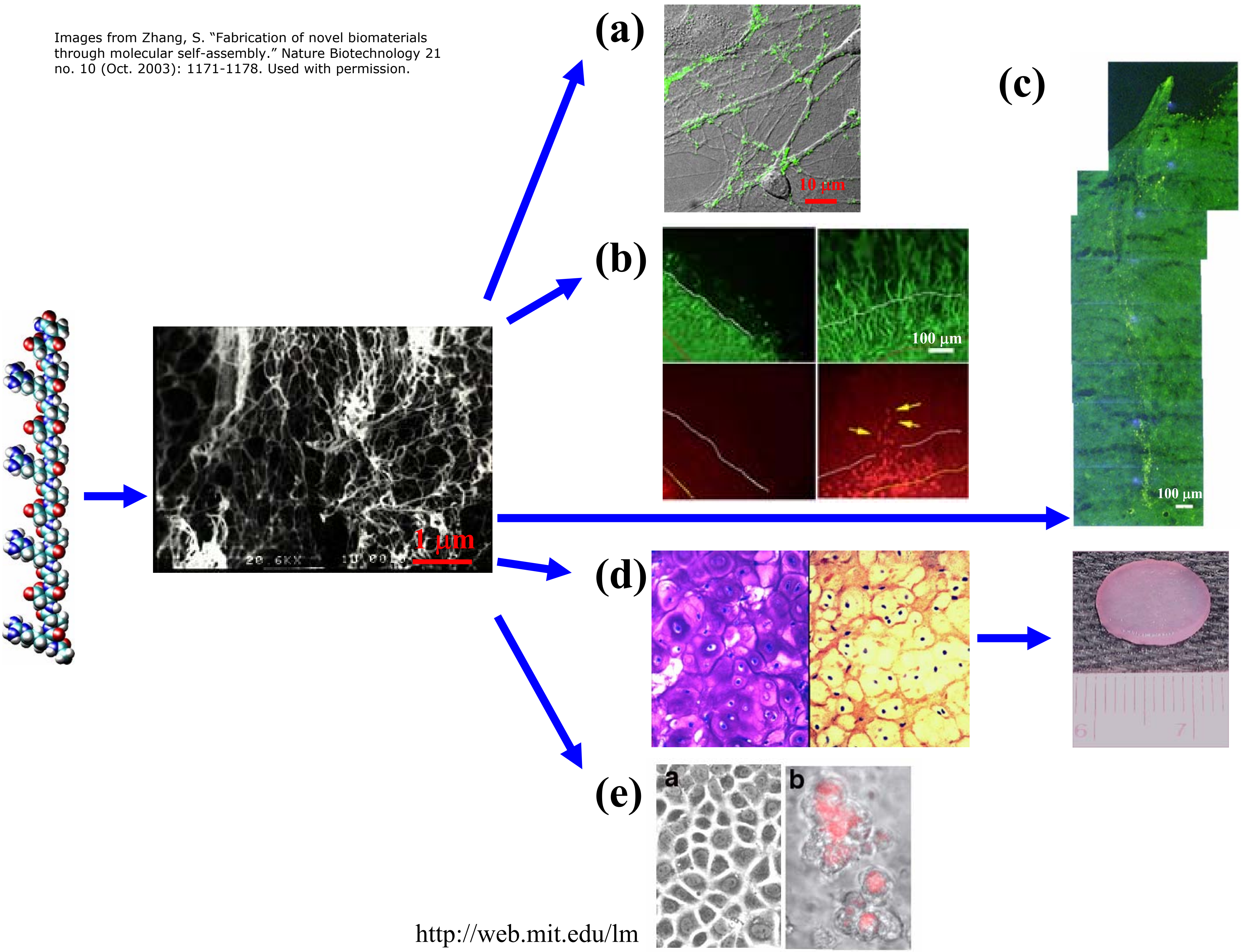
Outer sheet
(S24)

Double sheet

AFM image

http://web.mit.edu/lms/www

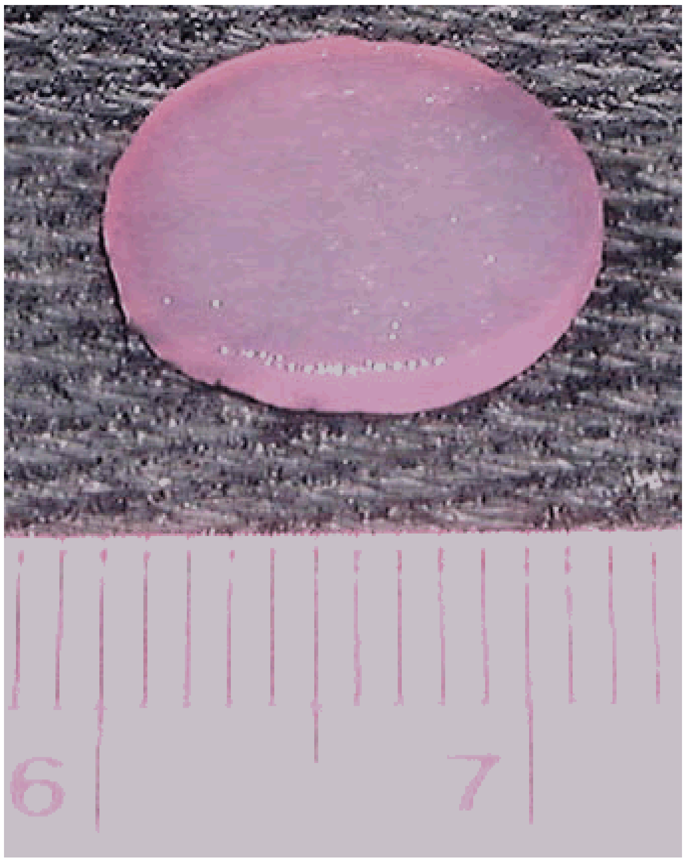
Images from Zhang, S. "Fabrication of novel biomaterials through molecular self-assembly." Nature Biotechnology 21 no. 10 (Oct. 2003): 1171-1178. Used with permission.




http://web.mit.edu/lm

# Chondrocytes encapsulated in KLD peptide scaffold

John Kisiday (Electric Engineering & Biological Engineering, MIT)




Source: Fig 1b in Kisiday, et al. "Self-assembling Peptide Hydrogel Fosters Chondrocyte Extracellular Matrix Production and Cell Division: Implications for Cartilage Tissue Repair." *PNAS* 99 (July 2002). Copyright 2002, National Academy of Sciences, U.S.A. Courtesy of National Academy of Sciences, U.S.A. Used with permission.

**50 μm**

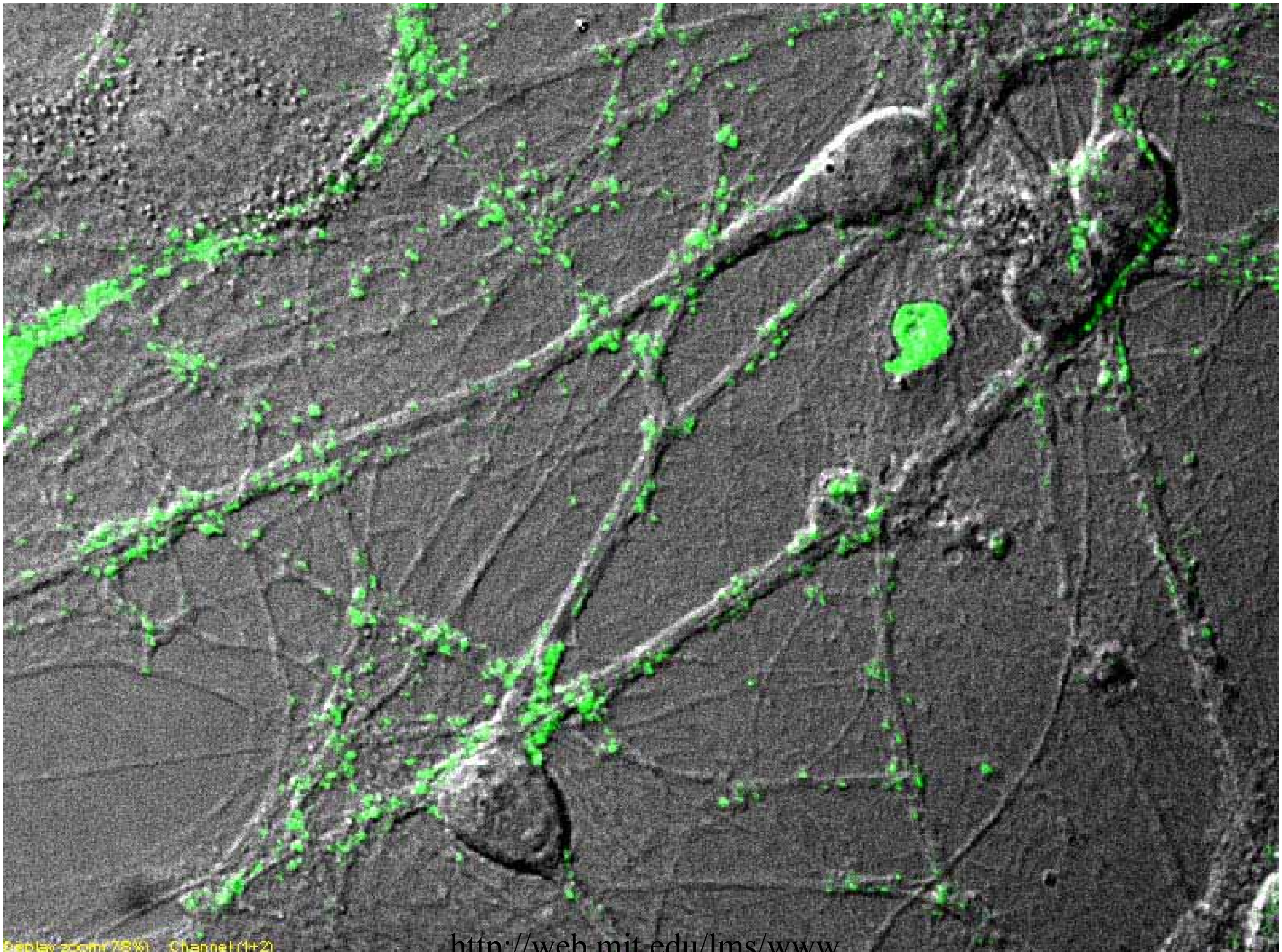
# *Culture brain hippocampal slice on peptide scaffold*

Photos removed for
copyright reasons.

**Carlos Semino**
**(Submitted)**

# ***Hippocampal Neurons form active connections on the self-assembling RAD16 peptide scaffold***

**Holmes, et al, June 2000**




Source: Figure 5a in Holmes, et al. "Extensive Neurite Outgrowth and Active Synapse Formation on Self-assembling Peptide Scaffolds." *PNAS* 97, no. 12 (June 6, 2000): 6728–6733. 

# *Peptide scaffold bridges the gap & repairs brain lesion*

**Rutledge Ellis-Behnke, et al., Brain & Cognitive Science, MIT**

Photos removed for
copyright reasons.

http://web.mit.edu/lms/www

# Systems Studied using Peptide Scaffolds

| Cell Types | Cell Lines | Animals |
|---|---|---|
| Mouse fibroblast | NIH-3T3 | Mouse |
| Chicken embryo fibroblast | CEF | Rat |
| Chinese hamster ovary | CHO | Hamsters |
| Human cervical carcinoma | Hela | Rabbits |
| Human osteosarcoma | MG63 | Goats |
| Human hepato-cellular carcinoma | HepG2 | Monkey* |
| Hamster pancreas | HIT-T15 | Horse* |
| Human embryonic Kidney | HEK293 | |
| Human neuroblastoma† | SH-SY5Y | |
| Rat pheochromocytoma† | PC12 | |
| Mouse cerebellum granule cells*† | | |
| Mouse & rat hippocampal cells*† | | |
| Human foreskin fibroblast* | | |
| Human epidermal keratinocytes* | | |
| Bovine chrondrocytes* | | |
| Bovine endothilial cells* | | |
| Rat liver stem cells* | | |
| Mouse cardiac myocytes* | | |
| Rat neural stem cells*† | | |
| Rat hippocampal neural tissue slice*† | | |

# Think3-D!

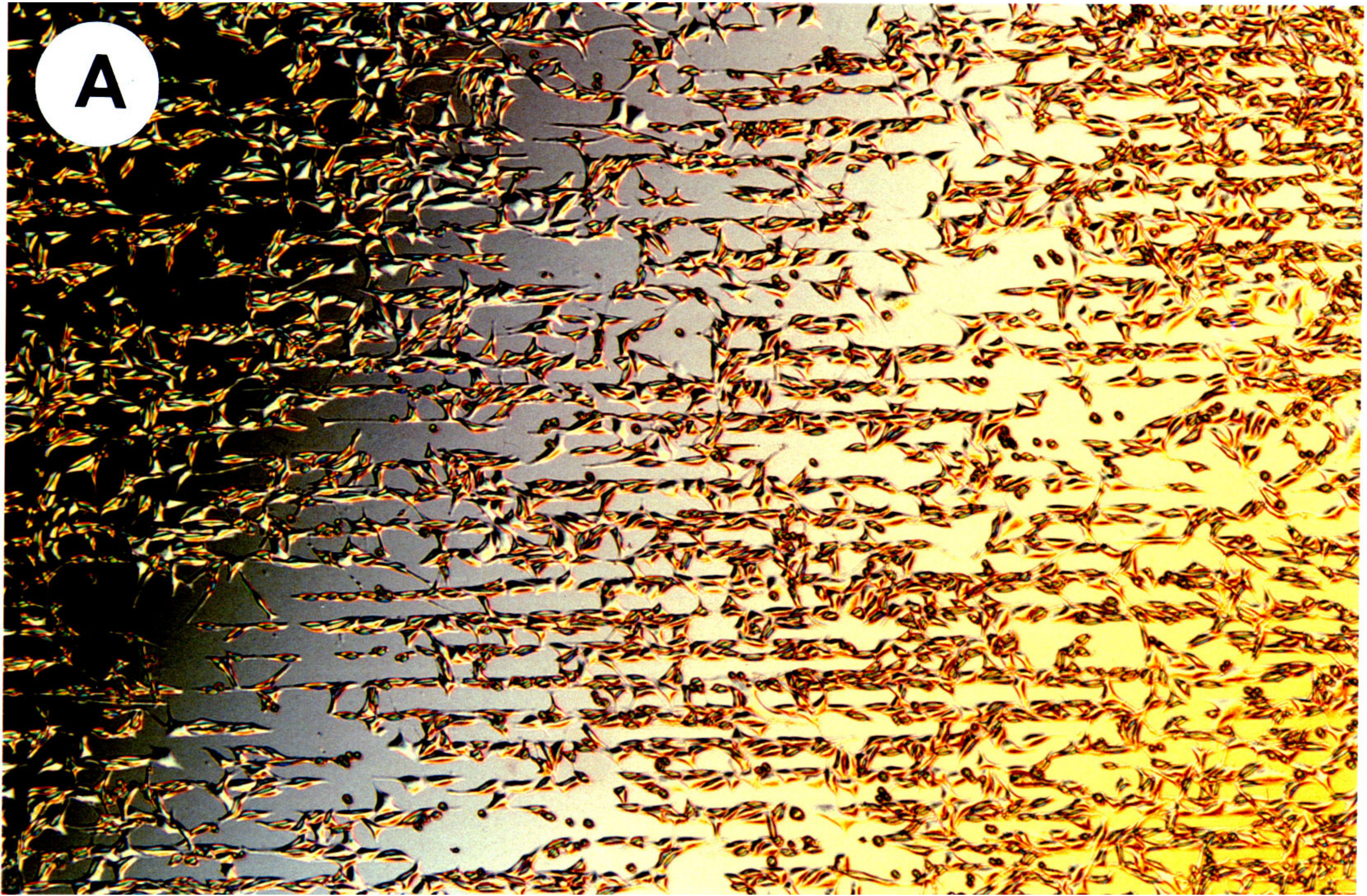
# ***Surface Self-assembling Peptide and $EG_6SH$***

## Zhang, et al, Biomaterials, Dec. 1999

## $EG_6SH$ 3 nm RADS Peptide 4 nm

Figure removed for
copyright reasons.

# Cell Tracks on the Peptide/EG Surface



# *Cells Stations and Tracks*

**Zhang, et al, Biomaterials, Dec. 1999**

Figure removed for
copyright reasons.

# ***Remarks and Perspectives***

**•Building from bottom-up for new Biological materials.**

**•Self-assembling peptides can serve as Nanoscale scaffolds.**

**•Peptides could be important as one of the components of the "Designed Materials".**

**• Many unexpected biological events and cell behaviors may be discovered when study cells in a 3-dimensional environment.**

http://web.mit.edu/lms/www

***In nature hybrid species are usually sterile, but in science the reverse is often true. Hybrid subjects are often astonishingly fertile, whereas if a scientific discipline remains too pure it usually wilts.***

**Francis Crick**

***What Mad Pursuit, 1988***

***Imagination is more important than knowledge.***

**Albert Einstein**

# *What do they have in common?*

| Made by Human | Made by Nature |
|---|---|
| **Machines** | **Molecular machines** |
| Transportation | Hemoglobin |
| Assembly lines | Ribosomes |
| Digital database | Nucleosomes |
| Copy machines | Polymerases |
| Bulldozer/Destroyer | Proteases/proteosome |
| Chain couplers | Ligases |
| Train control center | Centrosome |
| Train tracks | Actin filament network |
| Mail sorting machine | Protein sorting |
| Electric Fences | Membranes |
| Gates/keys & passes | Ion channels |
| Internet/www | Neuron synapse |