Fabricate Biological Nanomaterials

For Tissue Engineering

HST-535

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http://web.mit.edu/lms/www
Tissue Engineering requires 2 key ingredients:

- **Stem cells**
  - Embryonic stem cells
  - Adult stem cells

- **Biological scaffolds**
  - Polymer inert scaffolds
  - Designed functional biological scaffolds

http://web.mit.edu/lms/www
Self-assembly is ubiquitous in Nature

Each fish is about 5--50 centimeters in length

Photo courtesy of USGS.

http://web.mit.edu/lms/www
Molecular Self-assembly of Tetrameric Hemoglobin Polypeptides

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
- $\text{H}_2\text{O}$ mediated interactions (Water-mediated H-bonds)
Introducing: the self-assembling peptide nanofiber scaffold to culture, to grow and to study tissue cells in 3-dimensions: "Molecular Cement"

http://web.mit.edu/lms/www
Discovery of Peptide Nanofibers Scaffolds
Self-assembling Peptides Inspired from Nature

Found in a yeast protein, Zuotin


http://web.mit.edu/lms/www
Self-assembling Peptide Nanofibers

Scanning EM Image, EKA16-II

Self-assembling peptide nanofiber scaffold

RADA16-I
(SEM)

Biopolymers PGA/PLLA microfibers used as scaffold

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)

Marini, et al., NanoLetters, April, 2002

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

http://web.mit.edu/lms/www
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
http://web.mit.edu/lms/www
AFM & Cryo-TEM images of EFK8 nanofiber intermediate

Davide Marini, Mechanical Engineering
Marini, et al., NanoLetters, April, 2002

AFM    Cryo-TEM

Left-handed double helix

Two figures removed for copyright reasons.

http://web.mit.edu/lms/www
The molecular modeling & simulations
Marini, et al., NanoLetters, April, 2002

Diagrams removed for copyright reasons.

Axis view

19 nm

Inner sheet (S13)  Outer sheet (S24)  Double sheet  AFM image
http://web.mit.edu/lms/www

http://web.mit.edu/lm
Chondrocytes encapsulated in KLD peptide scaffold

John Kisiday (Electric Engineering & Biological Engineering, MIT)


50 µm

http://web.mit.edu/lms/www
Photos removed for copyright reasons.

Carlos Semino
(Submitted)

http://web.mit.edu/lms/www
Hippocampal Neurons form active connections on the self-assembling RAD16 peptide scaffold

Holmes, et al, June 2000

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

<table>
<thead>
<tr>
<th>Cell Types</th>
<th>Cell Lines</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse fibroblast</td>
<td>NIH-3T3</td>
<td>Mouse</td>
</tr>
<tr>
<td>Chicken embryo fibroblast</td>
<td>CEF</td>
<td>Rat</td>
</tr>
<tr>
<td>Chinese hamster ovary</td>
<td>CHO</td>
<td>Hamsters</td>
</tr>
<tr>
<td>Human cervical carcinoma</td>
<td>Hela</td>
<td>Rabbits</td>
</tr>
<tr>
<td>Human osteosarcoma</td>
<td>MG63</td>
<td>Goats</td>
</tr>
<tr>
<td>Human hepatocellular carcinoma</td>
<td>HepG2</td>
<td>Monkey*</td>
</tr>
<tr>
<td>Hamster pancreas</td>
<td>HIT-T15</td>
<td>Horse*</td>
</tr>
<tr>
<td>Human embryonic Kidney</td>
<td>HEK293</td>
<td></td>
</tr>
<tr>
<td>Human neuroblastoma†</td>
<td>SH-SY5Y</td>
<td></td>
</tr>
<tr>
<td>Rat pheochromocytoma†</td>
<td>PC12</td>
<td></td>
</tr>
<tr>
<td>Mouse cerebellum granule cells**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse &amp; rat hippocampal cells**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human foreskin fibroblast</td>
<td></td>
<td></td>
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<tr>
<td>Human epidermal keratinocytes</td>
<td></td>
<td></td>
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<tr>
<td>Bovine chondrocytes</td>
<td></td>
<td></td>
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<tr>
<td>Bovine endothelial cells</td>
<td></td>
<td></td>
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<tr>
<td>Rat liver stem cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse cardiac myocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat neural stem cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat hippocampal neural tissue slice**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Think 3-D!

http://web.mit.edu/lms/www
Surface Self-assembling Peptide and $EG_6SH$


$EG_6SH$ 3 nm  RADS Peptide 4 nm

Figure removed for copyright reasons.

http://web.mit.edu/lms/www
Cell Tracks on the Peptide/EG Surface
Cells Stations and Tracks


Figure removed for copyright reasons.

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

http://web.mit.edu/lms/www
### What do they have in common?

<table>
<thead>
<tr>
<th>Made by Human Machines</th>
<th>Made by Nature Molecular machines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transportation</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Assembly lines</td>
<td>Ribosomes</td>
</tr>
<tr>
<td>Digital database</td>
<td>Nucleosomes</td>
</tr>
<tr>
<td>Copy machines</td>
<td>Polymerases</td>
</tr>
<tr>
<td>Bulldozer/Destroyer</td>
<td>Proteases/proteosome</td>
</tr>
<tr>
<td>Chain couplers</td>
<td>Ligases</td>
</tr>
<tr>
<td>Train control center</td>
<td>Centrosome</td>
</tr>
<tr>
<td>Train tracks</td>
<td>Actin filament network</td>
</tr>
<tr>
<td>Mail sorting machine</td>
<td>Protein sorting</td>
</tr>
<tr>
<td>Electric Fences</td>
<td>Membranes</td>
</tr>
<tr>
<td>Gates/keys &amp; passes</td>
<td>Ion channels</td>
</tr>
<tr>
<td>Internet/www</td>
<td>Neuron synapse</td>
</tr>
</tbody>
</table>

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