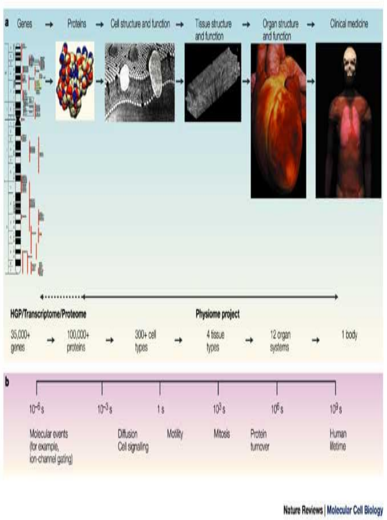
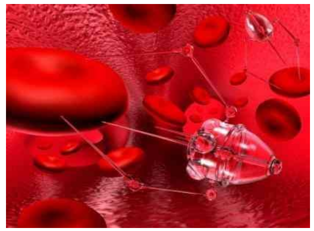
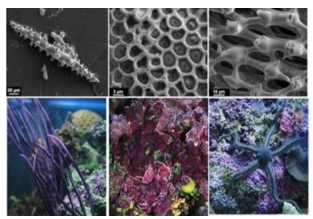
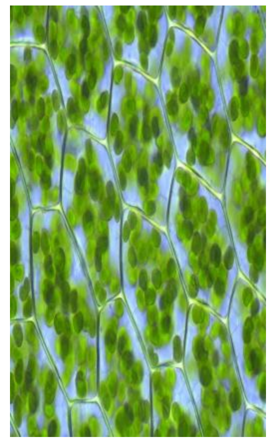
Nano-enabled Biological Tissues



COURTESY: Nature Reviews Molecular Cell Biology, 4, 237-243 (2003).

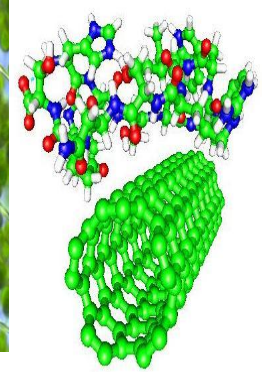


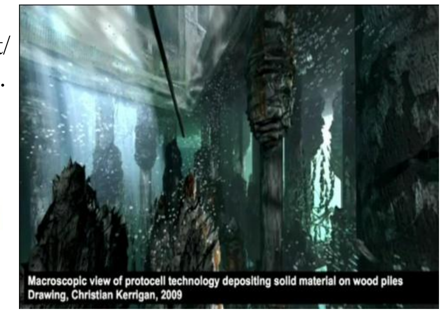


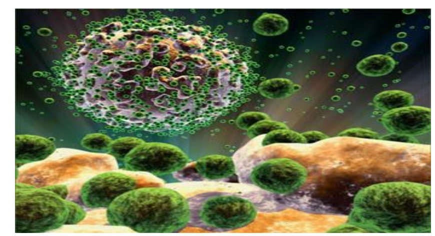


http://laegroup.ccmr.cornell.edu/

http://www.afs.enea.it/project/cmast/group3.php







COURTESY: http://library.thinkquest.org/05aug/00736/nanomedicine.htm

Your funding agency logo here

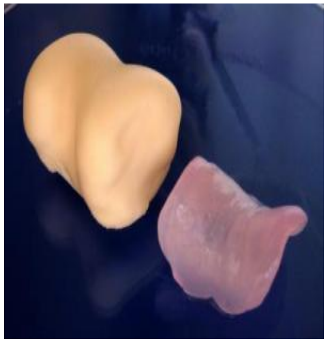
Your funding agency logo here

By Bradly Alicea

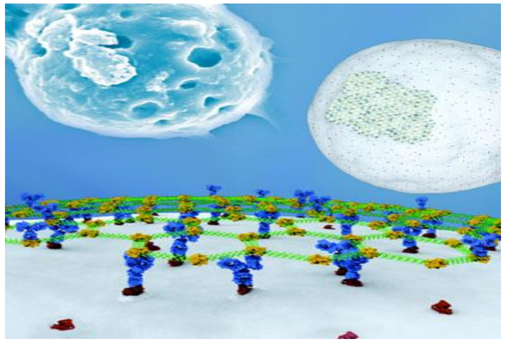
http://www.msu.edu/~aliceabr/

Presented to PHY 913 (Nanotechnology and Nanosystems, Michigan State University). October, 2010.

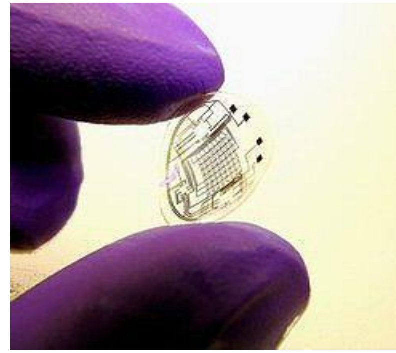
Nanoscale Technology Enables Complexity at Larger Scales…….



Self-assembled cartilage



Nano-scale biofunctional surfaces (cell membrane) http://www.nanowerk.com/spotlight/spotid=12717.php



Flexible electronics embedded in contact lens

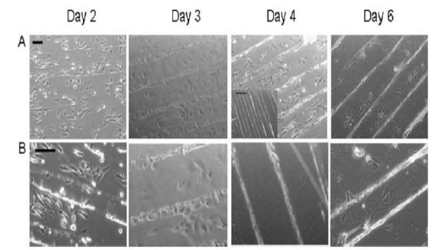
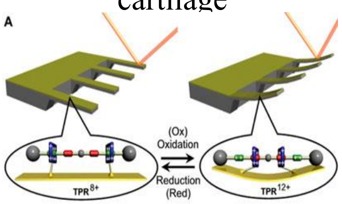
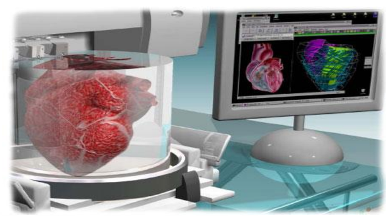


Fig. 3 Progression of cardiac organoid formation on HA patterned surfaces. (A) Images taken at 100 × . Day 4 inset image taken at 40 × illustrates several millimeter-long cardiac organoids. (B) Images taken at 200 × . Scale bars (A,B) 100 μm. Inset scale bar 1 mm

Formation (above) and function (below) of contractile organoids. Biomedical Microdevices, 9, 149–157 (2007).



DNA/protein sensor, example of BioNEMS device (left).



“Bioprinting” to construct a heart (left).

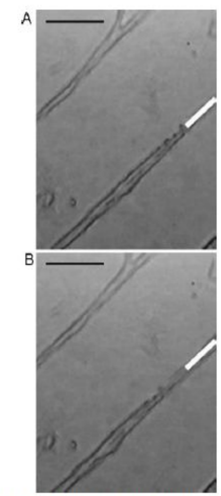
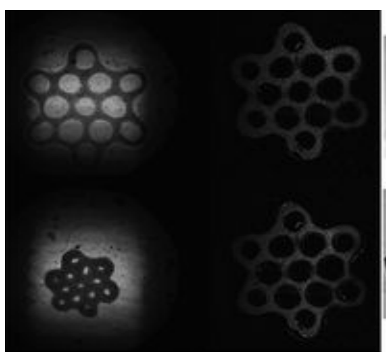
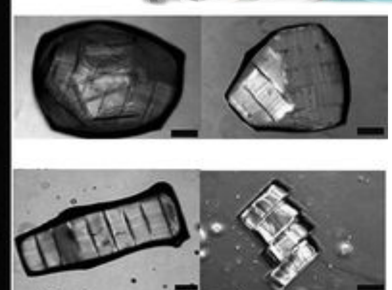


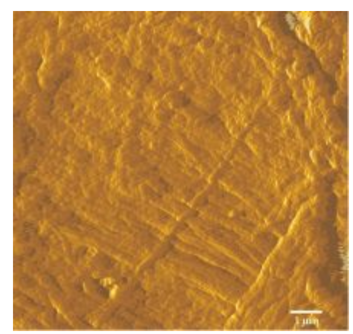
Fig. 4 Spontateneous contraction of an organoid after 6 days in culture. The white line indicates a position of the landmark at the beginning of contraction. The landmark shifts downward as the organoid contracts. (A) Frame taken at $t = 0$ (B) Frame taken at $t = 800$ ms, when the landmark has shifted by 38 μm. Scale bar 100 μm

Cells cultured in matrigel clusters





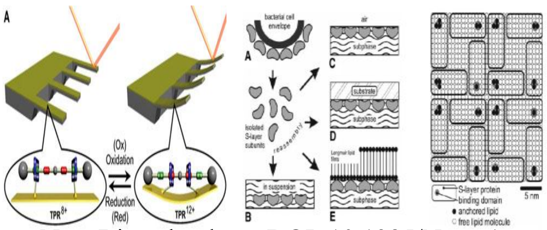
Guided cell aggregation. COURTESY: “Modular tissue engineering: engineering biological tissues from the bottom up”. Soft Matter, 5, 1312 (2009).



Self-organized collagen fibrils

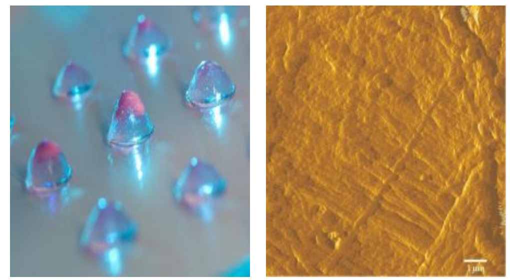
Role of Scale (Size AND Organization)

Single molecule monitoring and bio-functionalization

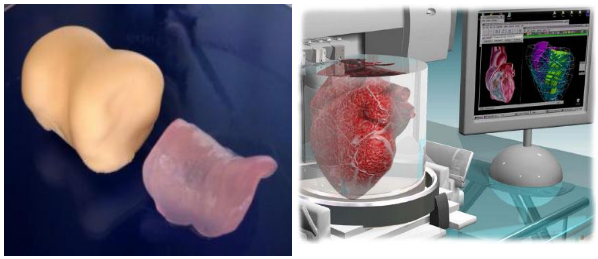


NanoBiotechnology, DOI: 10.1385/Nano:1:2:153 (2005).

Cell colonies and biomaterial clusters

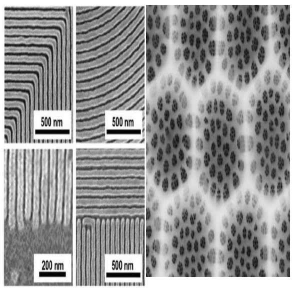


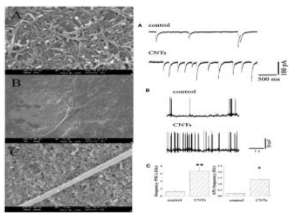
Self-assembled and bioprinted organs



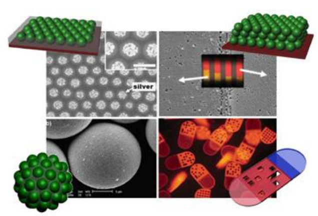
~ 1 nm	**10-100 nm**	**1-100 µm**	**1-100 mm**	**1-100 cm**	**+ 1m**

Nanopatterning and biofunctionalized surfaces



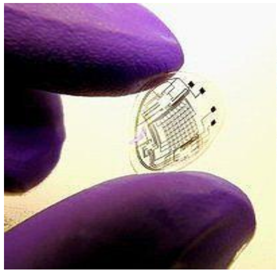


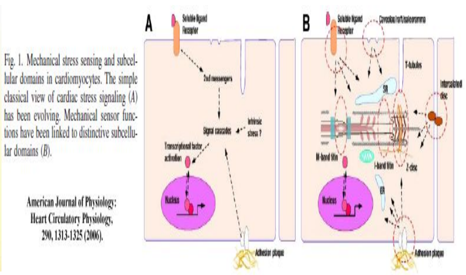
NanoLetters, 5(6), 1107-1110 (2005)



Soft Matter, 6, 1092-1110 (2010)

Embedded and hybrid bionic devices

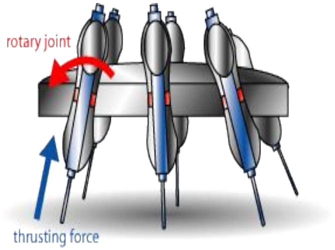




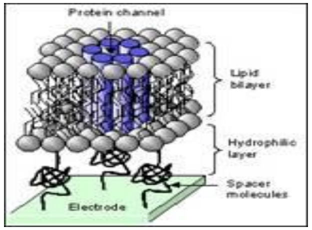
Ingredient I, Biomimetics/ Biocompatibility

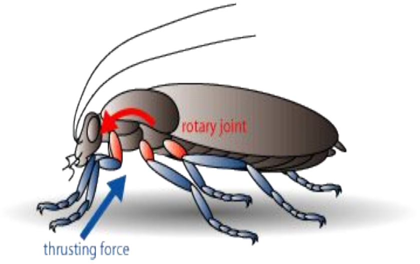
Biomimetics: engineering design that mimics natural systems.

Nature has evolved things better than humans can design them.



* can use biological materials (silks) or structures (synapses).





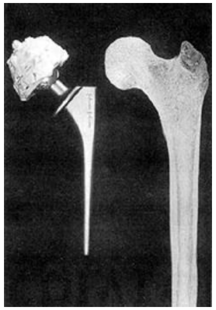
Biocompatibility: materials that do not interfere with biological function.

* compliant materials used to replace skin, connective tissues.

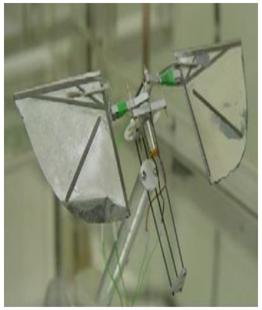
* non-toxic polymers used to prevent inflammatory response in implants.

Polylactic Acid Coating

Cyclomarin Source



Hydroxyapatite (Collagen)



Parylene (Smart Skin)

Artificial Skin, Two Approaches

Approximating cellular function:

"Tissue-Engineered Skin Containing Mesenchymal Stem Cells Improves Burn Wounds". Artificial Organs, 2008.

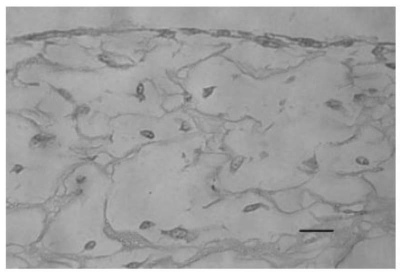


FIG. 2. Hematoxylin–eosin-stained histological section of mesenchymal stem cells (MSCs) grown on collagen-GAG scaffolds. Wavelike collagen bundles and of randomly scattered MSCs can be observed. Scale bars = 100 μm.

Stem cells better than synthetic polymers (latter does not allow for vascularization).

* stem cells need cues to differentiate.

* ECM matrix, "niche" important.

* biomechanical structure hard to approximate.

Approximating electrophysiology:

"Nanowire active-matrix circuitry for low-voltage macroscale artificial skin". Nature Materials, 2010.

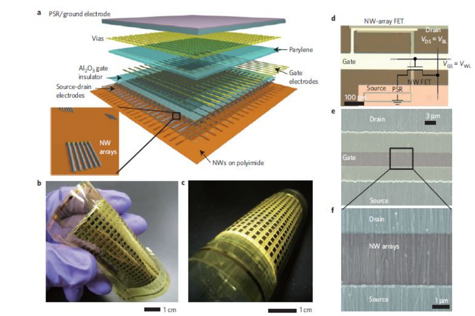
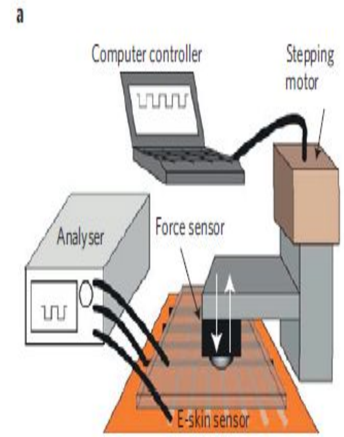


Figure 1 | Nanowire-based macroscale flexible devices. a, Schematic of the passive and active layers of NW e-skin (see Methods). **b**,**c**, Optical photographs of a fully fabricated e-skin device (7×7 cm^2 with a 19×18 pixel array) under bending (**b**) and rolling (**c**) conditions. **d**, Optical-microscope image of a single sensor pixel in the array, depicting a Ge/Si NW-array FET (channel length ~3 μm, channel width ~250 μm) integrated with a PSR. The circuit structure for the pixel is also shown. **e**,**f**, Scanning electron micrographs of a NW-array FET, showing the high degree of NW alignment and uniformity achieved by contact printing with a density of ~5 NWs μm^{-1}.

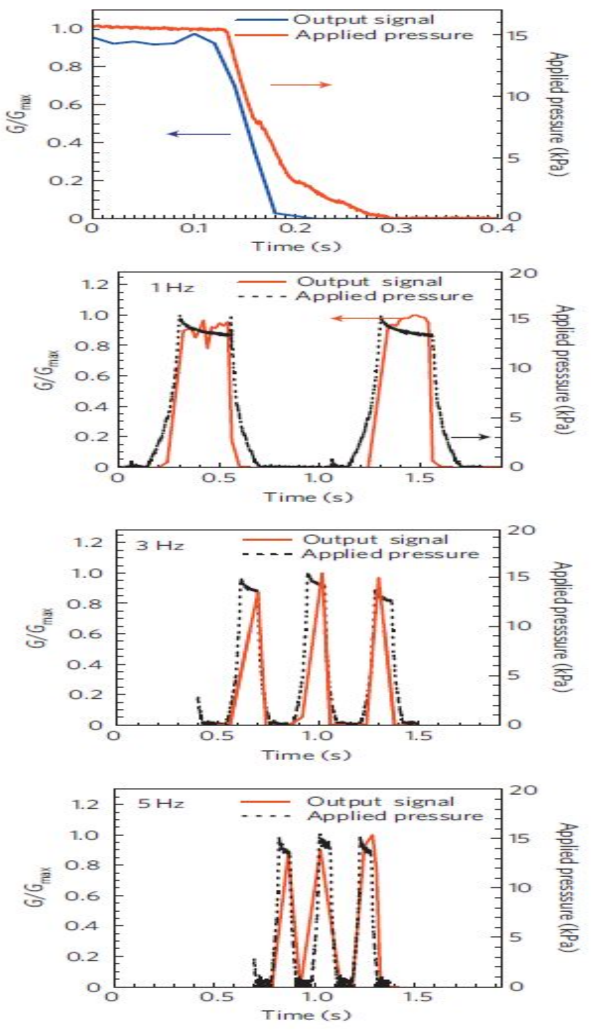


Skin has important biomechanical, sensory functions (pain, touch, etc).

* approximated using electronics (nanoscale sensors embedded in a complex geometry).

* applied force, should generate electrophysiological-like signal.

Artificial Skin – Response Characteristics



Results for stimulation of electronic skin:

Output signal from electronic skin, representation is close to pressure stimulus.

* only produces one class of sensory information (pressure, mechanical).

Q: does artificial skin replicate neural coding?

* patterned responses over time (rate-coding) may be possible.

* need local spatial information (specific to an area a few sensors wide).

* need for intelligent systems control theory at micro-, nano-scale.

Silk as Substrate, Two Approaches

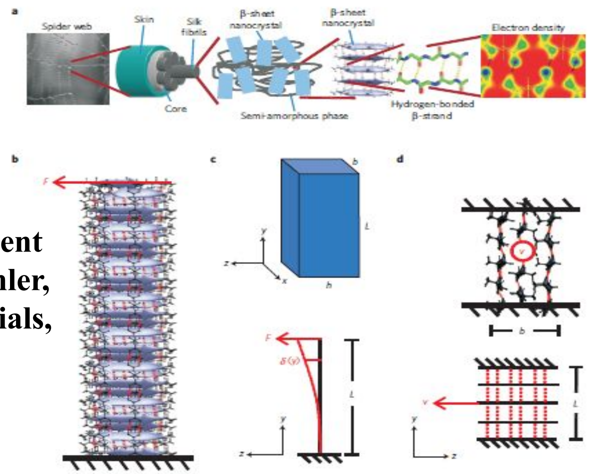
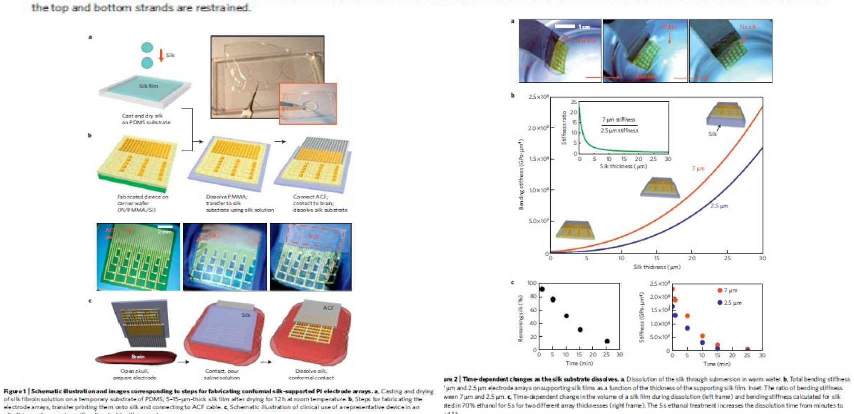


Figure 1 | Hierarchical structure of spider silk, simulation set-up and theoretical considerations. a, Schematic of the hierarchical spider silk structure that ranges from nano to macro. **a** displays key structural features of silk, including the electron density at the Angstrom scale, hydrogen bonded β-strands, β-sheet nanocrystals, a hetero-nanocomposite of stiff nanocrystals embedded in a softer semi-amorphous phase and silk fibrils, which assembles into macroscopic silk fibres. **b,** The atomistic structure of the silk β-sheet nanocrystal obtained from the Protein Data Bank (identification code 2slk) and replicated to build β-sheet nanocrystals of different sizes. In the first set of simulations, the β-sheet nanocrystal is subject to loading conditions similar to a cantilever beam with a constant tip loading, used to identify the bending rigidity and other structural properties. This loading mimics the characteristic lateral loading relevant to silk mechanics. **c,** Schematic representation of the β-sheet nanocrystal and definition of coordinates used here (upper part, where parameters *b* and *h* describe geometric parameters related to the number of sheets and the length of strands in the nanocrystal, and *L* is the size of the nanocrystal in the *y* direction). The lower part shows the geometry of the bending study and defines the displacement variable. **d,** Set-up for pull-out simulations, where to characterize fracture resistance of the nanocrystals, the central strand of the middle sheet is pulled out with constant velocity, while the top and bottom strands are restrained.

Nanoconfinement M. Buehler, Nature Materials, 9, 359 (2010)



Bio-integrated Electronics. J. Rogers, Nature Materials, 9, 511 (2010)

Nanoconfinement (Buehler group, MIT):
* confine material to a layer ~ 1nm thick (e.g. silk, water).

* confinement can change material, electromechanical properties.

Bio-integrated electronics (Rogers group, UIUC):
Silk used as durable, biocompatible substrate for implants, decays *in vivo:*
* spider web ~ steel (Young's modulus).

* in neural implants, bare Si on tissue causes inflammation, tissue damage, electrical interference.

* a silk outer layer can act as an insulator (electrical and biological).

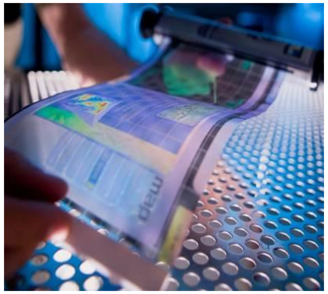
Ingredient II, Flexible Electronics

Q: how do we incorporate the need for compliance in a device that requires electrical functionality?

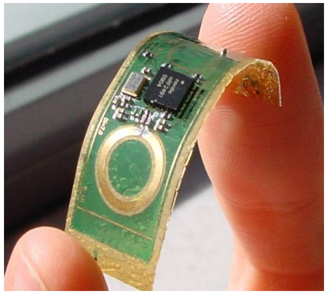
* tissues need to bend, absorb externally-applied loads, conform to complex geometries, dissipate energy.

A: Flexible electronics (flexible polymer as a substrate).

Flexible e-reader



Flexible circuit board



Nano version (Nano Letters, 3(10), 1353-1355 - 2003):

* transistors fabricated from sparse networks of nanotubes, randomly oriented.

* transfer from Si substrate to flexible polymeric substrate.

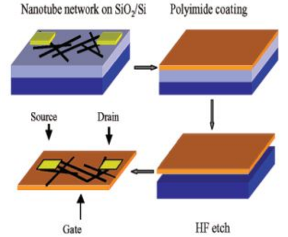


Figure 1. Schematic of the nanotube network transfer process. Networks are grown on oxide-coated silicon substrates, and metal contacts are deposited. Next, a polymer film is applied to the substrate. When the silicon oxide is etched away, the polymer film and the nanotube network lift off the substrate.

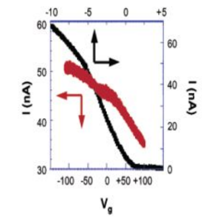
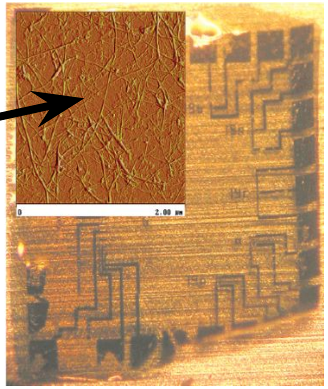


Figure 3. Current vs gate voltage for networks on a flexible polymer substrate (red) and on a rigid substrate (black), showing field-effect transistor operation in the two cases. The transistor on–off ratio of the latter is $\sim 10^4$.

Nano Letters, 3(10), 1353-1355 (2003)



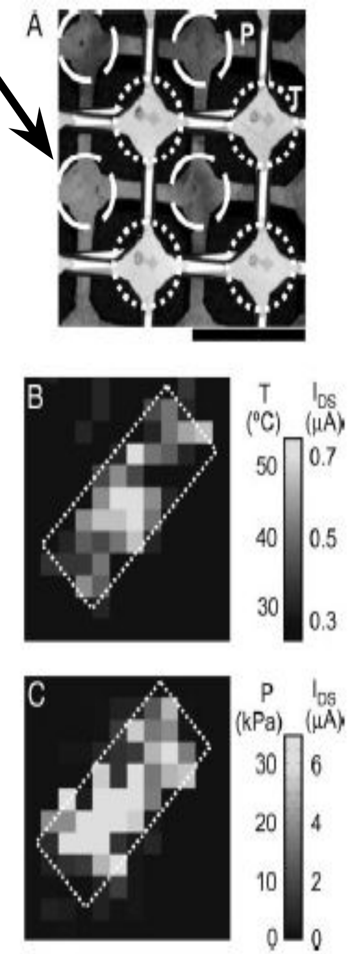
Sparse network of NTs.

E-skin for Applications

Organic field effect transistors (OFETs):
* use polymers with semiconducting properties.

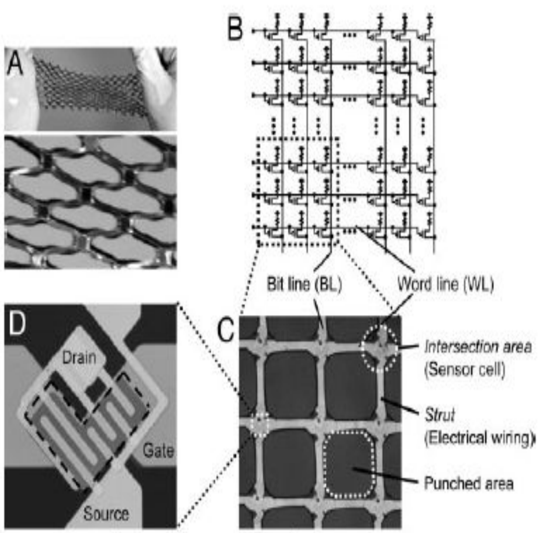
Embedded array of pressure and thermal sensors

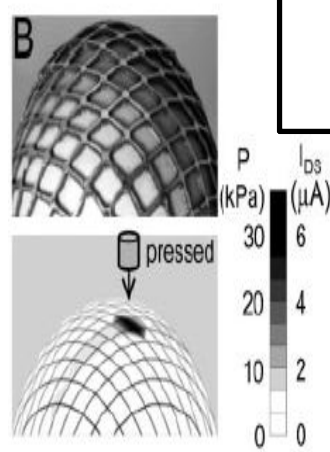
PNAS, 102(35), 12321–12325 (2005).



Thin-film Transistors (TFTs):
* semiconducting, dielectric layers and contacts on non-Si substrate (e.g. LCD technology).

* in flexible electronics, substrate is a compliant material (skeleton for electronic array).





PNAS, 102(35), 12321–12325 (2005).

Conformal network of pressure sensors

Create a bendable array of pressure, thermal sensors.

Integrate them into a single device (B, C – on right).

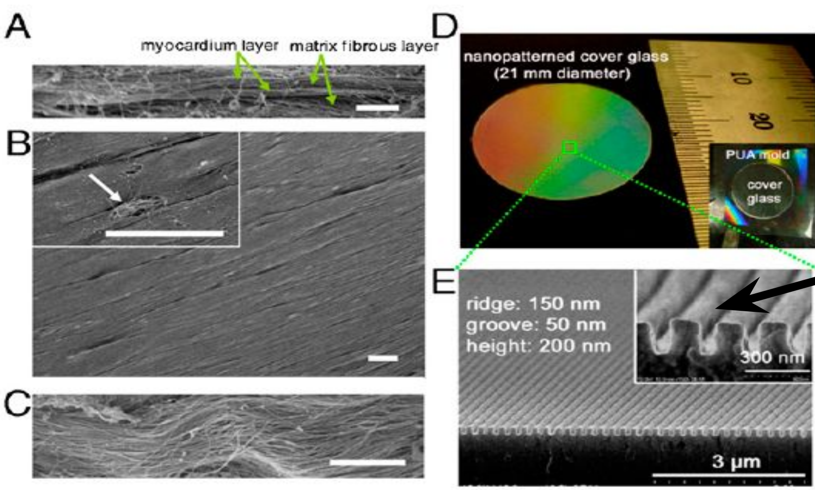
Ingredient III, Nanopatterning

Q: how do we get cells in culture to form complex geometries?

We can use nanopatterning as a substrate for cell monolayer formation.

* cells use focal adhesions, lamellapodia to move across surfaces.

* migration, mechanical forces an important factor in self-organization, self-maintenance.



Gratings at nanoscale dimensions

PNAS 107(2), 565 (2010)

Alignment and protrusions w.r.t nanoscale substrate

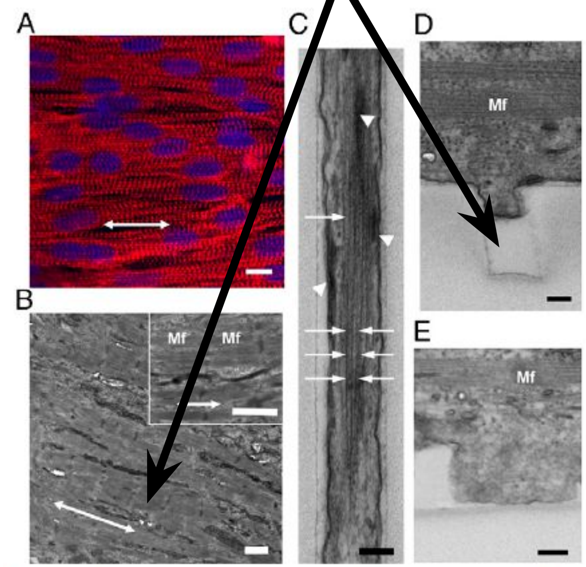


Fig. 3. Cell and cytoskeleton alignment and striations. (*A*) Immunofluorescent images of sarcomeric α-actinin (in red) of NRVMs cultured on the ANFS. Cell nuclei are shown in blue. (*B*) Cross-sectional TEM images of the engineered myocardial tissue grown on the ANFS showing aligned Mf with elongated sarcomeres. Double-headed arrows in (*A*) and (*B*) denote the direction of anisotropic nanopatterns consisting of ridges and grooves. (*C*) An enlarged view of actin bundles (white arrows) and focal adhesions (dark and thick lines indicated by white arrowheads) preferentially formed in parallel to the individual ridges and grooves of the ANFS. (*D*–*E*) Representative cross-sectional view of the PEG sidewalls showing the lower extent of cell protrusion into (*D*) a 400-nm-wide groove than of that into (*E*) an 800-nm-wide groove. [Scale bar: 10 µm in (*A*); 1 µm in (*B*); 200 nm in (*C*–*E*).]

MWCNTs as Substrate for Neurons

Multi-Wall CNT substrate for HC neurons: Nano Letters, 5(6), 1107-1110 (2005).

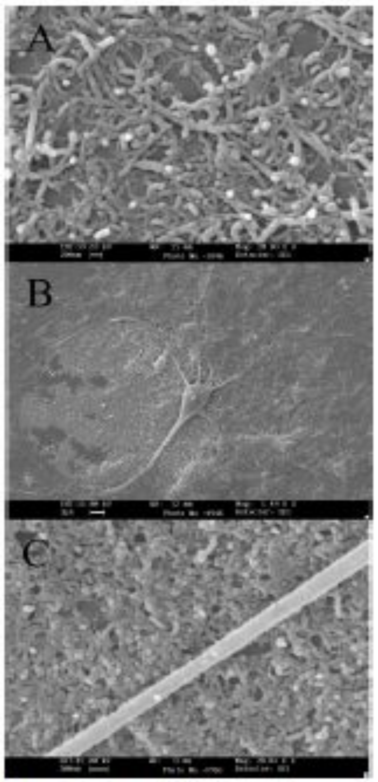


Figure 1. Purified multiwalled carbon nanotubes (MWNT) layered on glass are permissive substrates for neuron adhesion and survival. (A) Micrographs taken by the scanning electron microscope showing the retention on glass of MWNT films after an 8-day test in culturing conditions. (B) Neonatal hippocampal neuron growing on dispersed MWNT after 8 days in culture. The surface structure, composed of films of MWNT and peptide-free glass, allows neuron adhesion. Dendrites and axons extend across MWNT, glia cells, and glass. The relationship between dendrite and MWNT is very clear in the image in (C), were a neurite is traveling in close contact to carbon nanotubes.

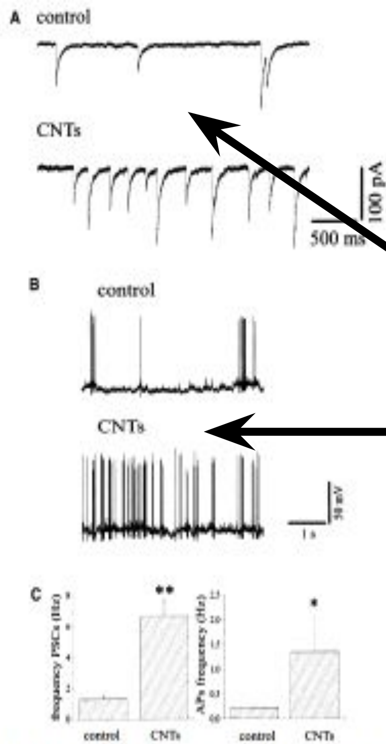
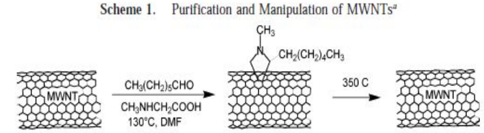


Figure 2. CNT substrate increases hippocampal neurons spontaneous synaptic activity and firing. (A) Spontaneous synaptic currents (PSCs) are shown in both control (top tracings) and in cultures grown on CNT substrate (bottom tracings). Note the increase in PSCs frequency under the latter condition. Recordings were taken after 8 days in culture. (B) Current clamp recordings from cultured hippocampal neurons in control (top tracings) and CNT growth conditions (bottom tracings). Spontaneous firing activity is greatly boosted in the presence of CNT substrates. (C) Histogram plots of PSCs- (left) and APs- (right) frequency in control and CNT cells; note the significant increase in the occurrence of both events when measured in CNT cultures. $^{**}P < 0.0001$ and $^{*}P < 0.05$.

Scheme 1. Purification and Manipulation of MWNTs[a]



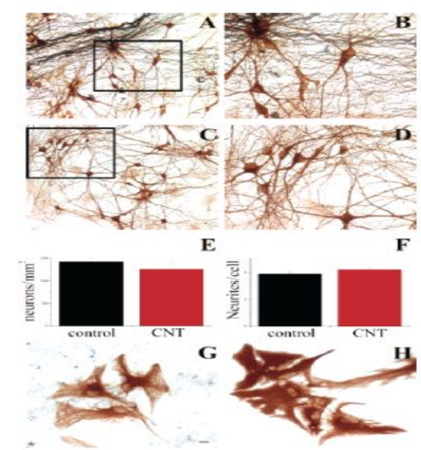
[a] The MWNTs were first functionalized and purified, then deposited on a glass substrate and heated to 350 °C, a process that eliminates the organic part, leaving intact the carbon network.

CNTs functionalized, purified, deposited on glass (pure carbon network desired).

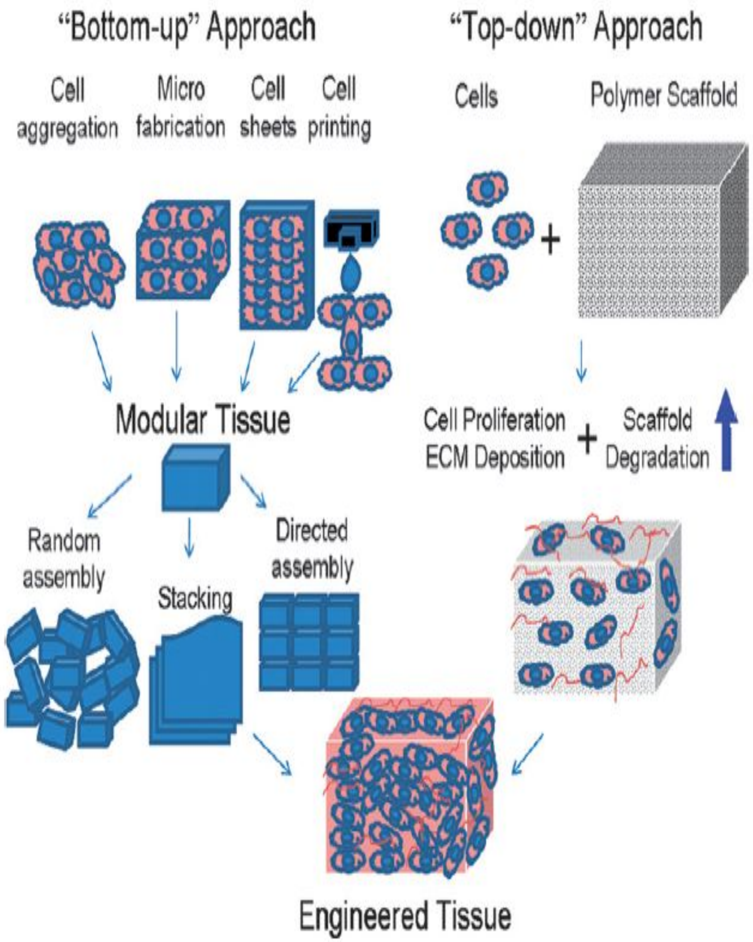
Improvement in electrophysiology: IPSCs (A) and patch clamp (B).

Neuronal density similar between CNTs and control.

* increase in electrical activity due to gene expression, ion channel changes in neuron.

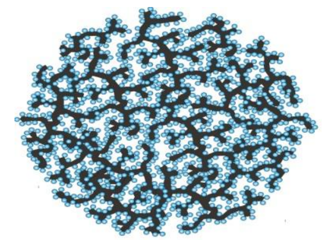


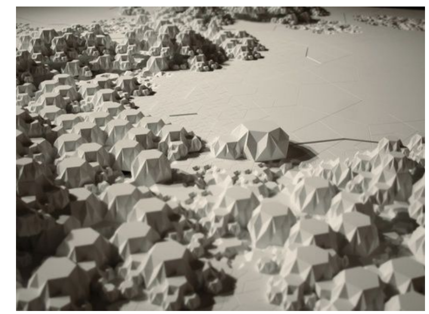
Bottom-up vs. Top-down Approaches



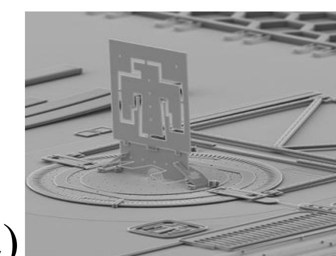
Soft Matter, 5, 1312–1319 (2009).

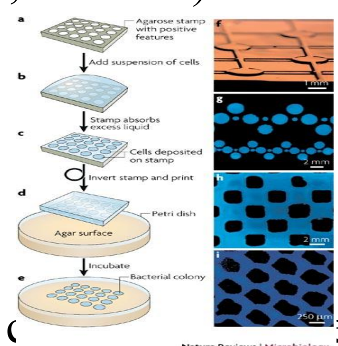
Theoretically, there are two basic approaches to building tissues:





1) bottom-up: molecular self-assembly (lipids, proteins), from individual components into structures (networks, micelles).



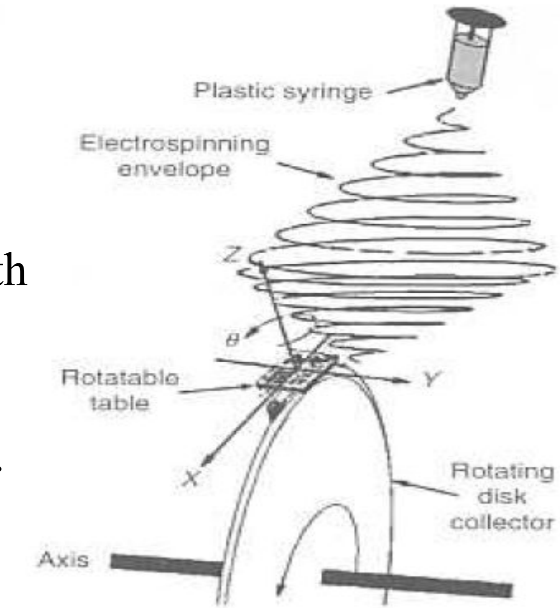


Nature Reviews Microbiology 5, 209-218 (2007).

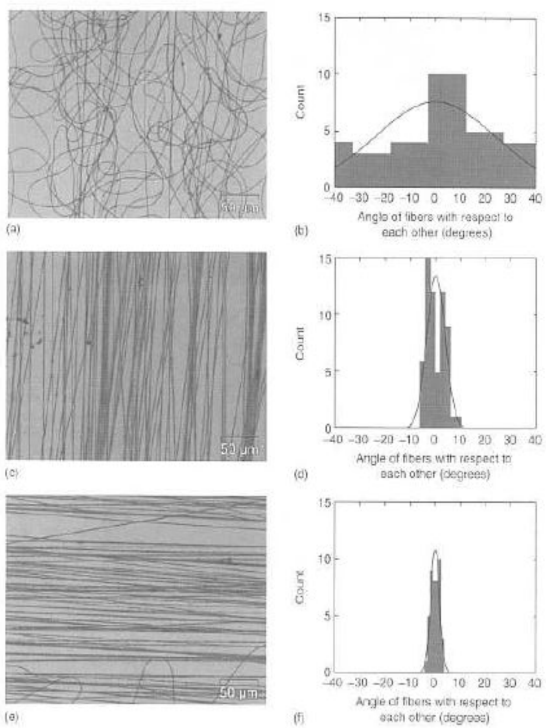
2) … upon a patterned substrate (CNTs, oriented ridges, microfabricated scaffolds).

Top-down approach: Electrospinning

Align nanofibers using electrostatic repulsion forces (review, see Biomedical Materials, 3, 034002 - 2008).



Contact guidance theory:
Cells tend to migrate along orientations associated with chemical, structural, mechanical properties of substrate.



Left: "Nanotechnology and Tissue Engineering: the scaffold". Chapter 9.

Right: Applied Physics Letters, 82, 973 (2003).

Electrospinning procedure:

* fiber deposited on floatable table, remains charged.

* new fiber deposited nearby, repelled by still-charged, previously deposited fibers.

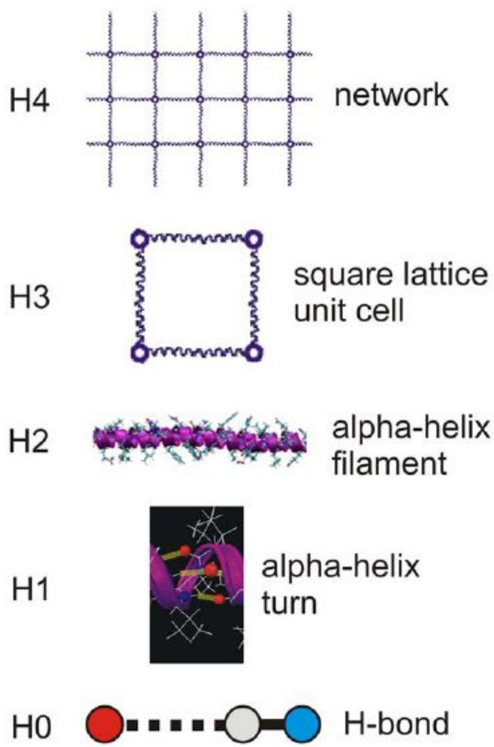
* wheel stretches/aligns fibers along deposition surface.

* alignment of fibers ~ guidance, orientation of cells in tissue scaffold.

Bottom-up approach: Molecular Self-assembly

Protein and peptide approaches commonly used.

Protein approach – see review, Progress in Materials Science, 53, 1101–1241 (2008).



Hierarchical Network Topology, MD simulations. PLoS ONE, 4(6), e6015 (2009).

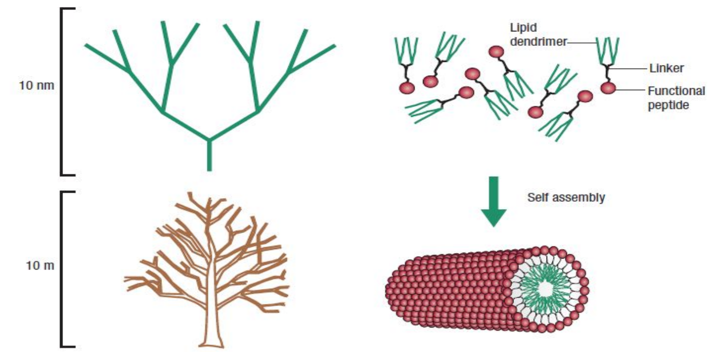


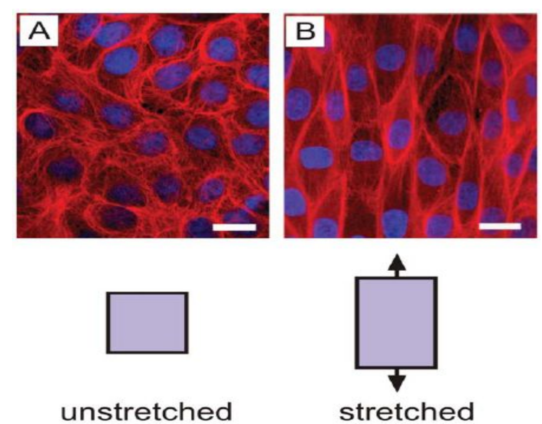
Figure 1 Dendrimers are tree-like molecules that have repeatedly branched structures. The combination of a functional peptide with dendritic lipid groups enables nanoparticles with controlled shapes and sizes to be assembled when the molecules are dissolved in water. The resulting assemblies have a hydrophobic lipid core (green) and a biologically active hydrophilic peptide coating (red).

Nature Nanotechnology, 3, 8 (2008).

α-helix protein networks in cytoskeleton withstand strains of 100-1000%.

* synthetic materials catastrophically fail at much lower values.

* due to nanomechanical properties, large dissipative yield regions in proteins.

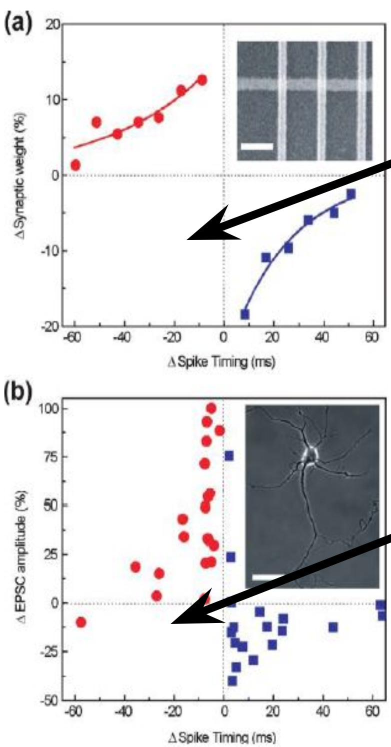


Filament network, *in vivo*. PLoS ONE, 4(6), e6015 (2009).

Additional Tools: Memristor

Memristor: information-processing device (memory + resistor, Si-based) at nanoscale.

* conductance incrementally modified by controlling change, demonstrates short-term potentiation (biological synapse-like).



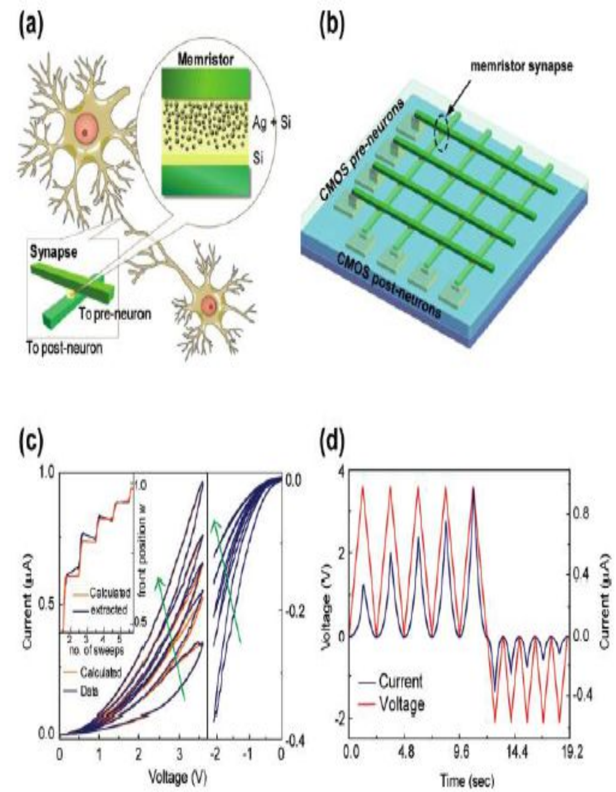
Memristor response

Biological Neuronal response

Learning = patterned (time domain) analog modifications at synapse (pre-post junction).

Array of pre-neurons (rows), connect with post-neurons (columns) at junctions.

* theory matches experiment!



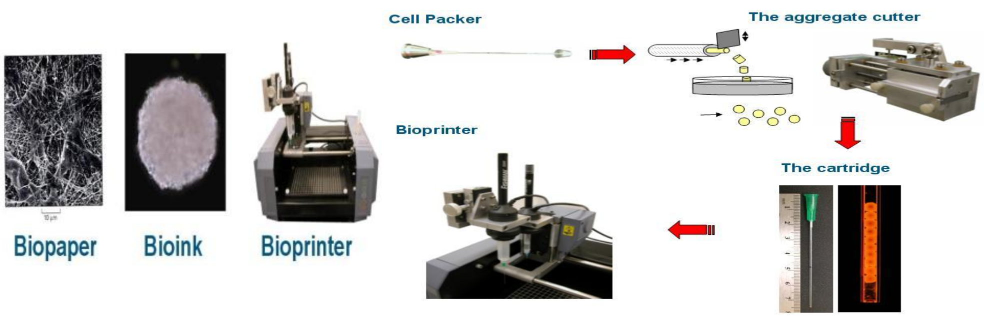
Nano Letters, 10, 1297–1301 (2010).

Nano Letters, 10, 1297–1301 (2010).

Additional Tools: Bioprinting

Bioprinting: inkjet printers can deposit layers on a substrate in patterned fashion.

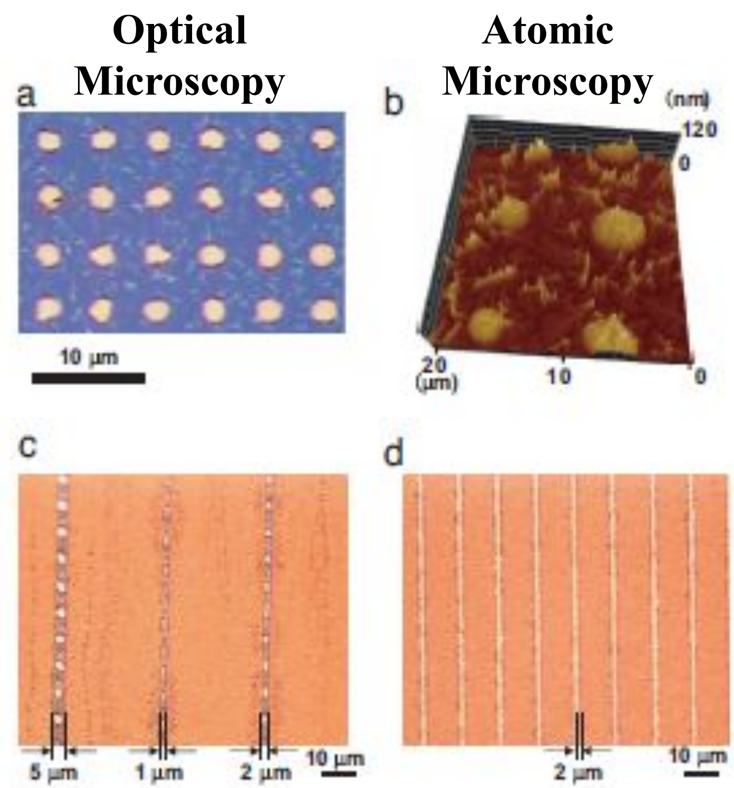
* 3D printers (rapid prototypers) can produce a complex geometry (see Ferrari, M., “BioMEMS and Biomedical Nanotechnology”, 2006).



Sub-femtoliter (nano) inkjet printing:
* microfabrication without a mask.

* amorphous Si thin-film transistors (TFTs), conventionally hard to control features smaller than 100nm.

* p- and n-channel TFTs with contacts (Ag nanoparticles) printed on a substrate.



PNAS, 105(13), 4976 (2008).

Conclusions

Nano can play a fundamental role in the formation of artificial tissues, especially when considering:

* emergent processes: in development, all tissues and organs emerge from a globe of stem cells.

* merging the sensory (electrical) and biomechanical (material properties) aspects of a tissue.

Advances in nanotechnology might also made within this problem domain.

* scaffold design requires detailed, small-scale substrates (for mechanical support, nutrient delivery).

* hybrid protein-carbon structures, or more exotic “biological” solutions (reaction-diffusion models, natural computing, Artificial Life)?