# Polymer Nanofibers in Medicine, Biotechnology & Engineering


## Seeram Ramakrishna

**Professor & Dean of Engineering**
**seeram@ nus.edu.sg**


*ICNT, Nov 7-11, 2004*

# Outline

- **Motivation for Polymer Nanofibers**
- **Quest for Polymer Nanofibers**
- **Electrospinning - State of the Art**
- **Affinity Membranes**
- **Tissue Engineering**

# Micron-Fibers

| Natural Fibers | Density [g/cm3] | Modulus (GPa) | Strength (MPa) | Application |
|---|---|---|---|---|
| Cotton | 1.54 | 9 ~ 13 | 400 ~ 660 | Clothing |
| Wool | 1.32 | 1 ~ 3 | 120 ~ 200 | Clothing |
| Silk | 1.33 | 6 ~ 12 | 350 ~ 470 | Clothing |
| Linen | 1.5 | 18 ~ 31 | 650 ~ 750 | Clothing |

| Fibers | Density [g/cm3] | Modulus (GPa) | Strength (MPa) | Application |
|---|---|---|---|---|
| Carbon (PAN) | 1.80 ~ 1.90 | 230 ~ 700 | 2000 ~ 7200 | Concrete reinforcing material, Sports items, Airplane |
| Carbon (Pitch) | 1.40 ~ 2.18 | 335 ~ 840 | 1000 ~ 3500 | Concrete reinforcing material, Sports items, Airplane |
| E-Glass | 2.51 | 69~72 | 3450 ~ 3790 | |
| S-Glass | 2.49 | 86~90 | 4590 ~ 4830 | |

# Micron-Fibers

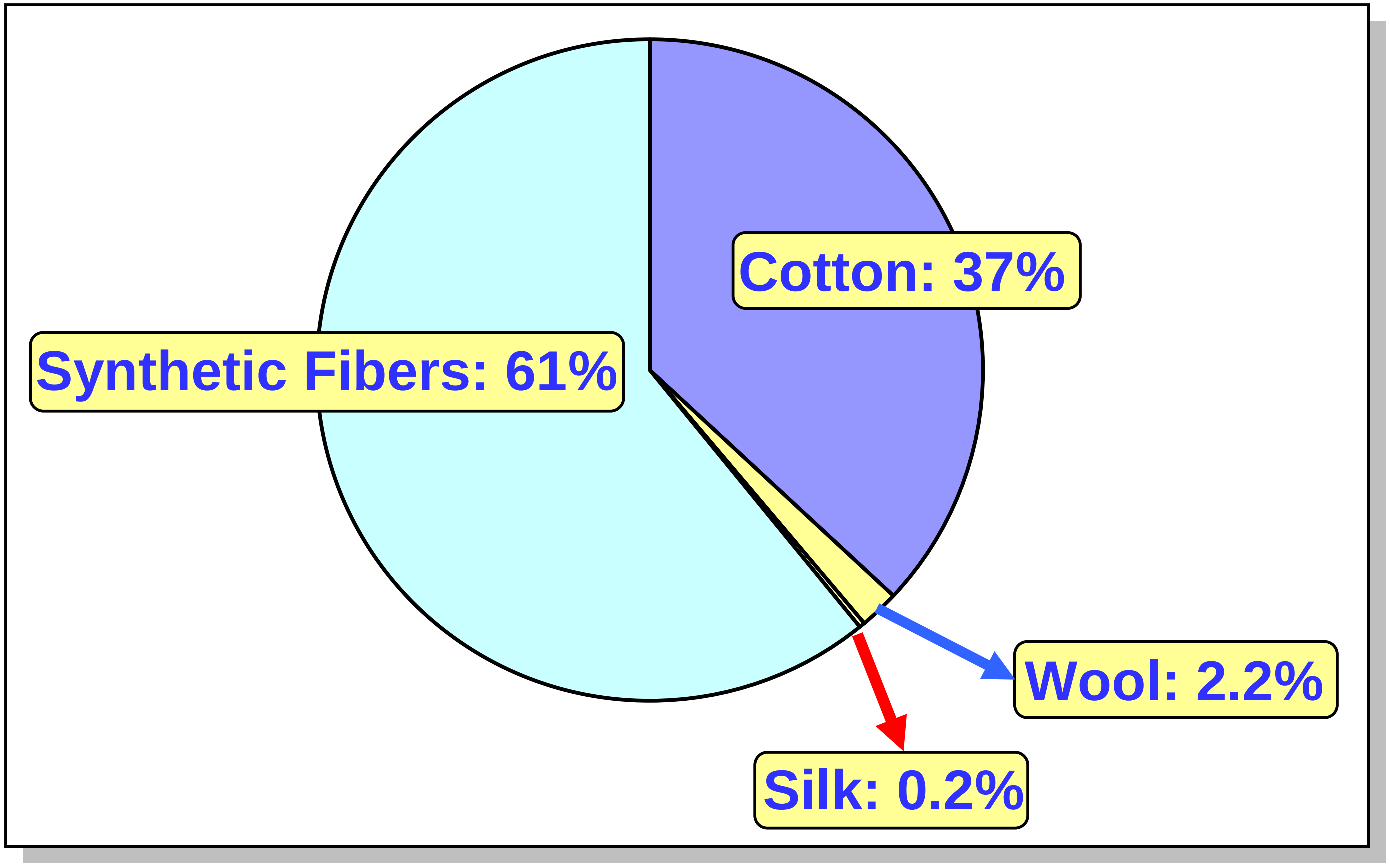
NUS National University of Singapore

| Polymer Fibers | Density [g/cm3] | Modulus (GPa) | Strength (MPa) | Application |
|---|---|---|---|---|
| Rayon (cellose) | 1.51 | 8.6 ~ 11 | 230 ~ 300 | Clothing, Towel |
| Acetate | 1.32 | 3.4 ~ 5.3 | 145 | Cigarett Filter, Clothing |
| Vinylon | 1.26 ~ 1.3 | 6.8 ~ 10 | 330 ~ 450 | Rubber and Concrete Reinforcement |
| Nylon 66 | 1.14 | 3.0 ~ 5.2 | 500 ~ 650 | Seawall bag, Clothing, Tire Coad, Fishing net, Carpet |
| PVC | 1.39 | 3.6 ~ 6.1 | 330 ~ 460 | Clothing |
| polyester | 1.38 | 11 ~ 20 | 530 ~ 733 | Clothing |
| polyacryl | 1.15 | 3.9 ~ 8.6 | 360 ~ 570 | Clothing, Mortar and Asphalt Reinforcement |
| polypropylene | 0.91 | 3.2 ~ 9.6 | 370 ~ 600 | Concrete Reinforcement, Sanitary Items |
| polyurethane | 1.0 ~ 1.3 | ---------- | 58 ~ 130 | Clothing |
| Aramid (para-type) | 1.39 ~ 1.45 | 56 ~ 150 | 2400 ~ 3500 | Protecting cloth, Flak jacket, Rope, Tire code, Aerospace materials |
| UHMW-polyethelyne | 0.97 ~ 0.98 | 70 ~ 175 | 2200 ~ 4800 | Rope, Fishing Yarn, Fishing Net |
| PEEK | 1.37 ~ 1.42 | 8.8 ~ 10 | 750 ~ 840 | Filter, Tire code, Belt |
| polyphenylenesulfide (PPS) | 1.34 ~ 1.36 | 3 ~ 8 | 540 ~ 660 | Filter, Insulating material |
| polyimide | 1.41 | 4 | 470 | Filter, Heat resistant cloth |
| PLLA | 1.27 | 5 ~ 8 | 460 ~ 690 | Fishing yarn, Fishing net, Plant net |
| PCL | 1.14 | 1.1 ~ 2.3 | 770 ~ 870 | Golf tee, Plastics bag, |
| poly(butylene succinate) | 1.26 | 1.9 ~ 3.2 | 620 ~ 740 | Food package film.Food tray, Sanitary items |

# World Fiber Consumption

NUS National University of Singapore

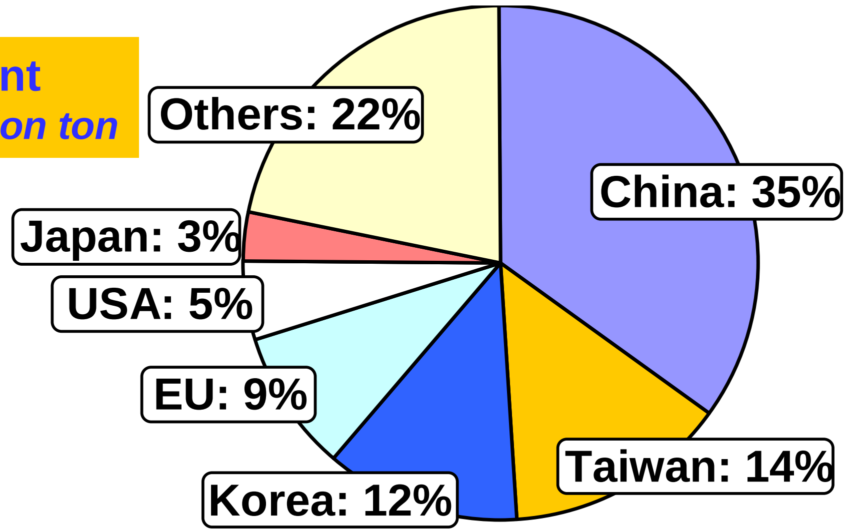
***Total amount = 58 million tons* in Year 2003**



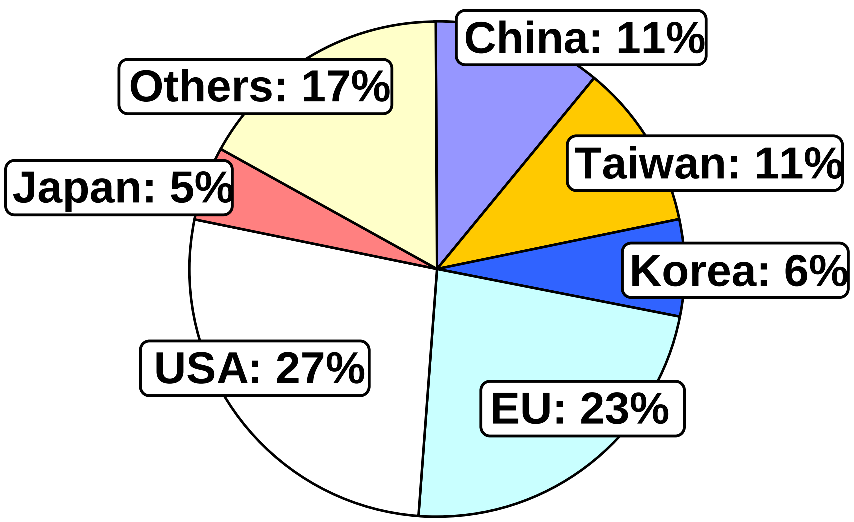

*Ref. Japan Chemical Fiber Association (http://www.jcfa.gr.jp)*

# World Fiber Production

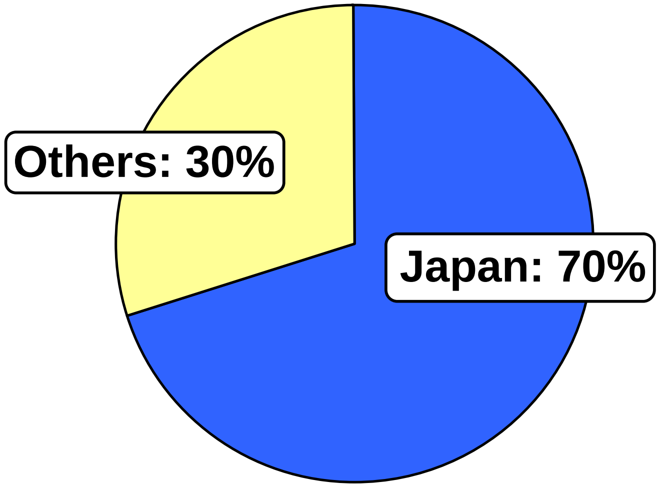
**Polyester Filament**
*Total amount = 11 million ton*




**Nylon Filament**
*Total amount = 3.7 million ton*

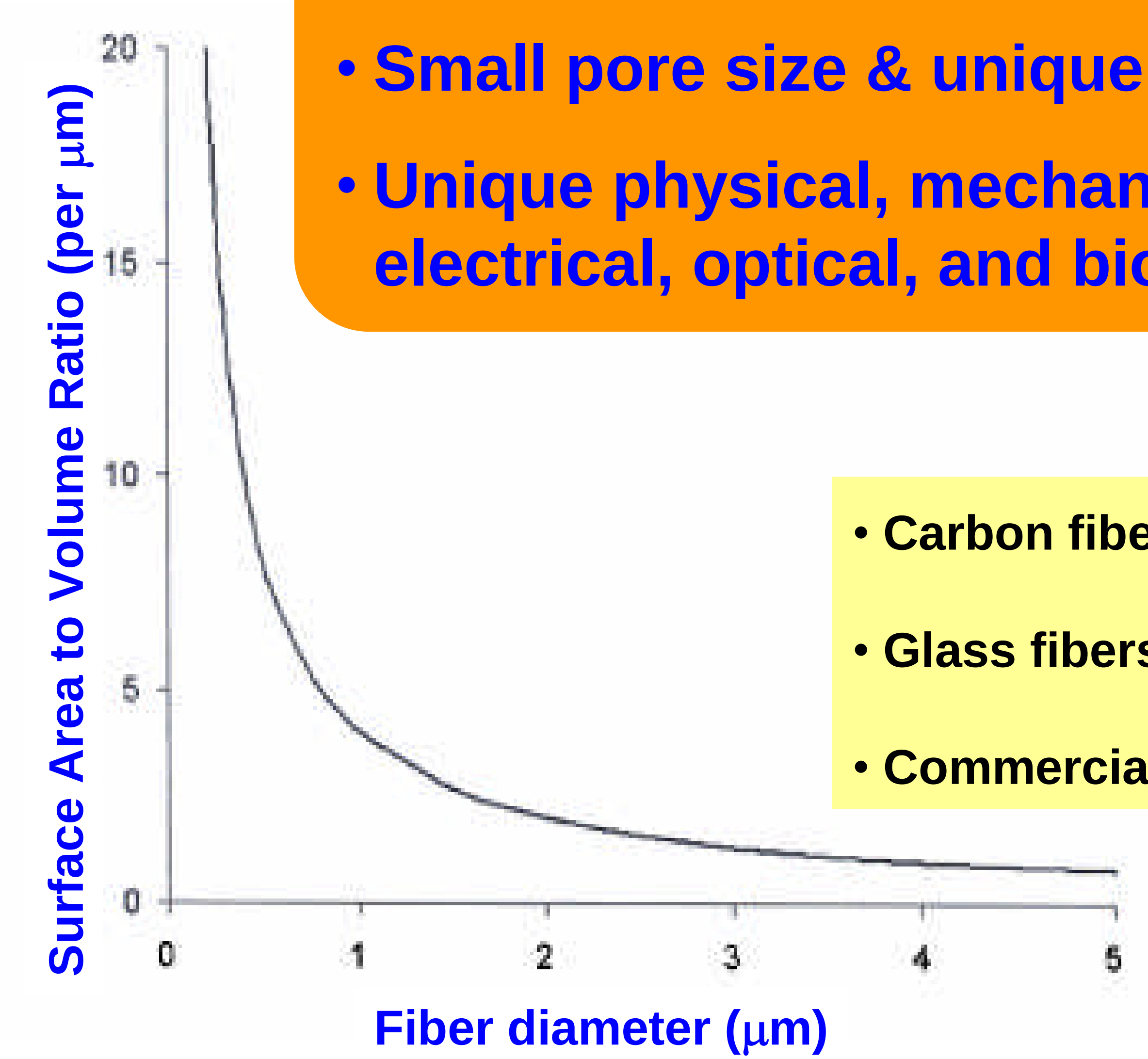



**Carbon FIber**
*Total amount = 14,000 ton*



# Why Nanofibers ?

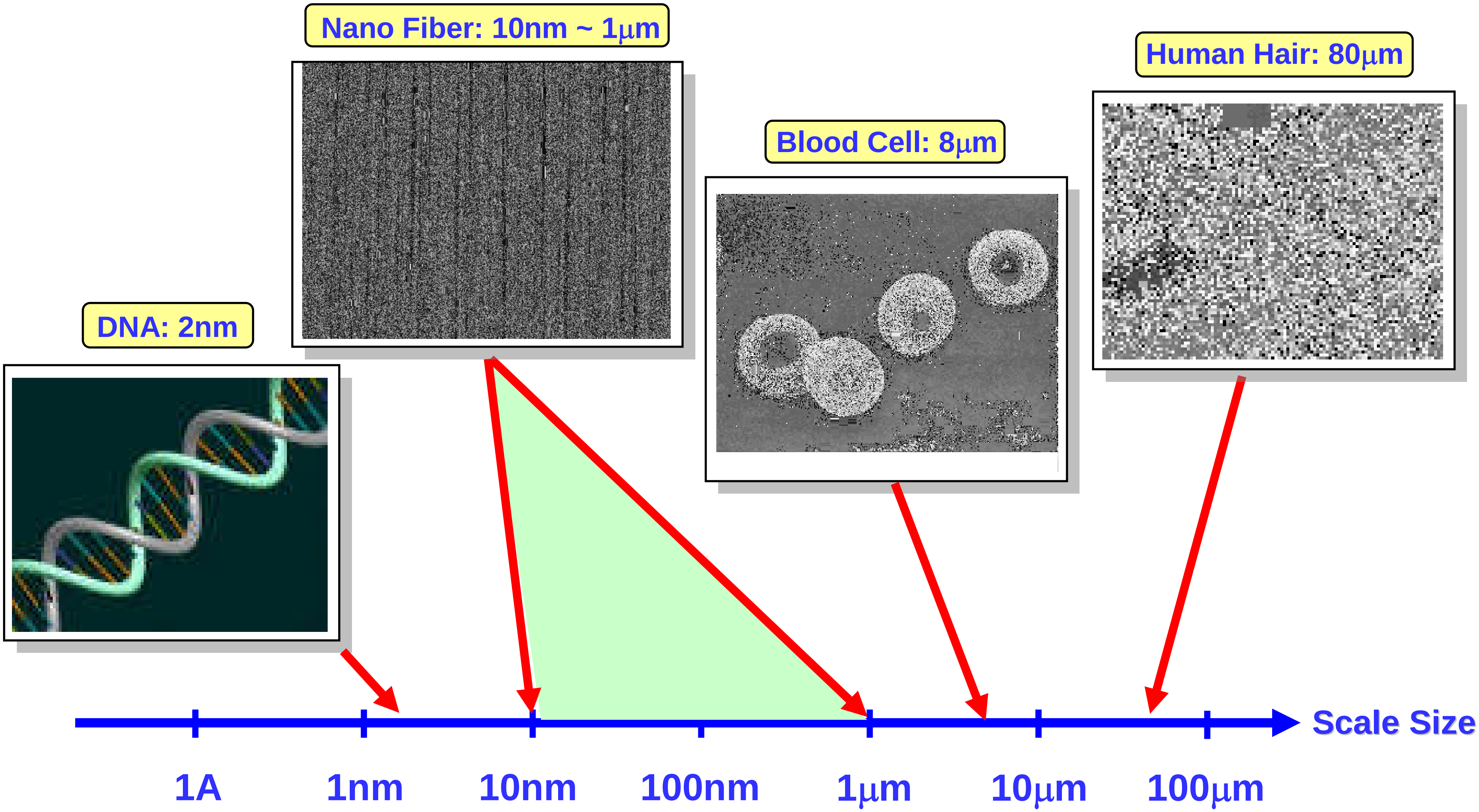
- Large surface area to volume ratio
- Small pore size & unique structures
- Unique physical, mechanical, chemical, electrical, optical, and biological properties




- Carbon fibers: 6 μm
- Glass fibers: 10 μm
- Commercial polymer fibers: >10 μm

*ICNT, Nov 7-11, 2004*

# Size Comparison



# Applications

NUS National University of Singapore

## Defense & Security

Material Reinforcement
Chemical & Biological Protection

Electronic Noses and Tongues

## Environmental Eng. & Biotechnology

Membranes, Sensors

## APPLICATIONS

## Bioengineering

Tissue Engineering,
Tissue Repair, & Drug Delivery

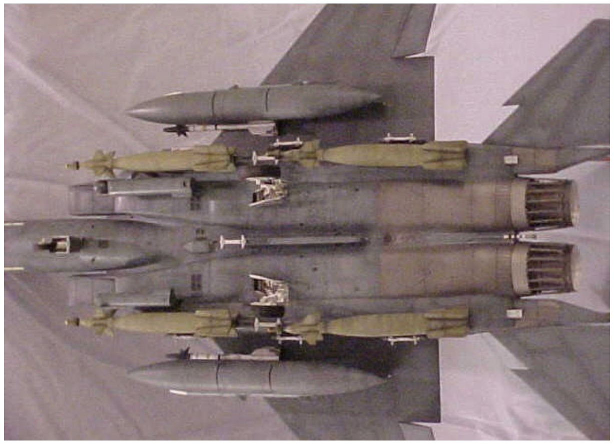
Conductors, Fuel Cell, High-Energy Density Battery, Micro-Sensors, Micro Hydrogen-Storage Devices, Field Emission Display, Photo-Electronic Devices.

## Energy & Electronics

# Defense & Security

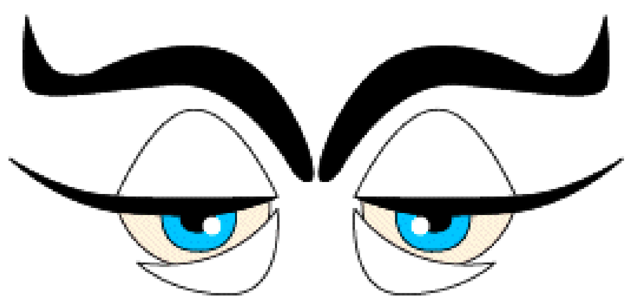
NUS National University of Singapore

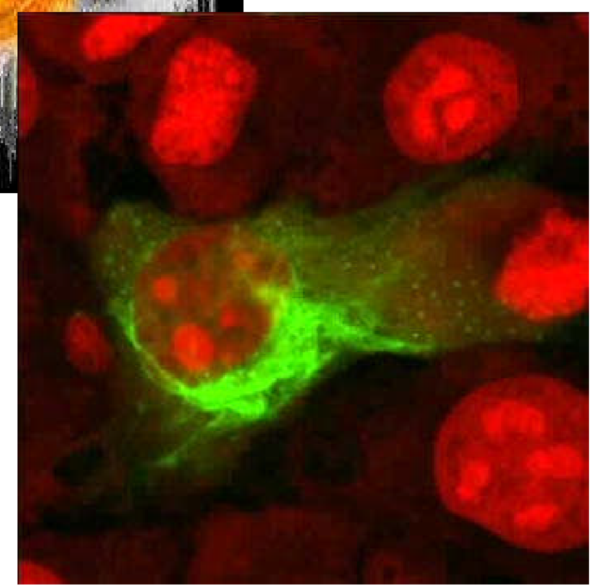
**Terrorism;**
**Natural Disasters**



# Defense & Security

**Protective Clothing that selectively capture Chemical (gases & radioactive compounds) and Biological (bacterial spores & viruses) contaminants**



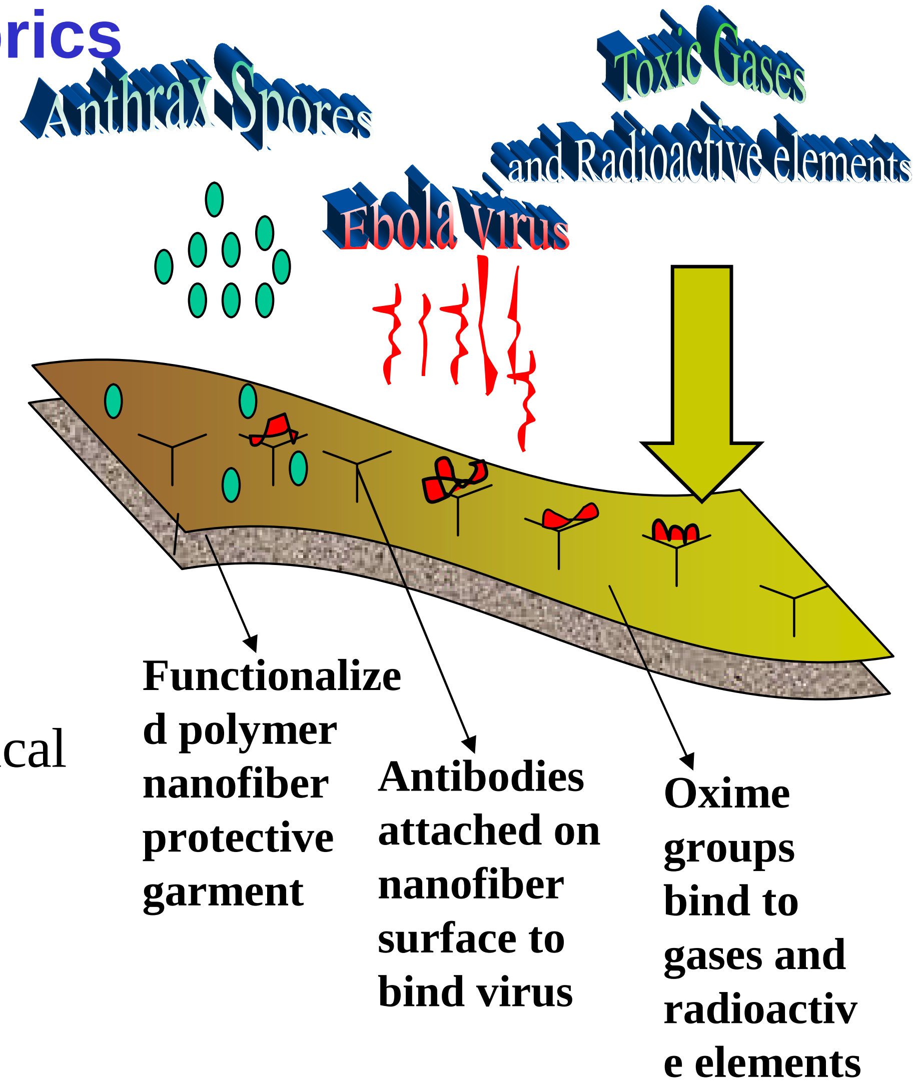
# Defense & Security

## Stop & Neutralize; Stop & Kill Fabrics

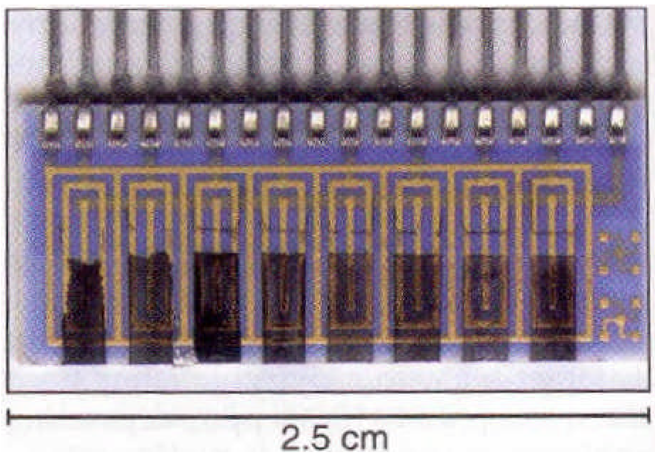
**Size Exclusion-** Functionalized polymer nanofibers act as a molecular sieve and prevent entry of bacteria

**Chemical Affinity-** Oxime functionality reacts to hydrolyze chemical toxins and helps to bind radioactive contaminants too

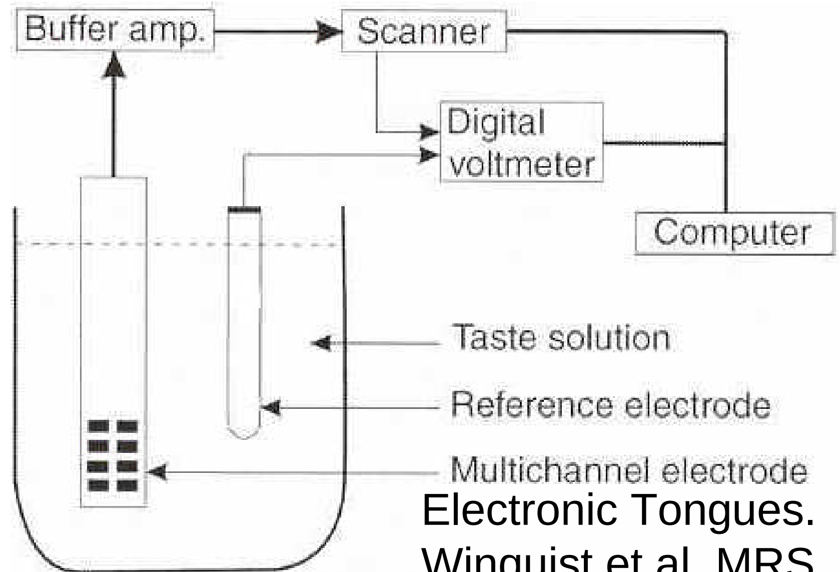
**Molecular Affinity-** Proteins present on nanofiber surface will selectively bind to biological contaminants



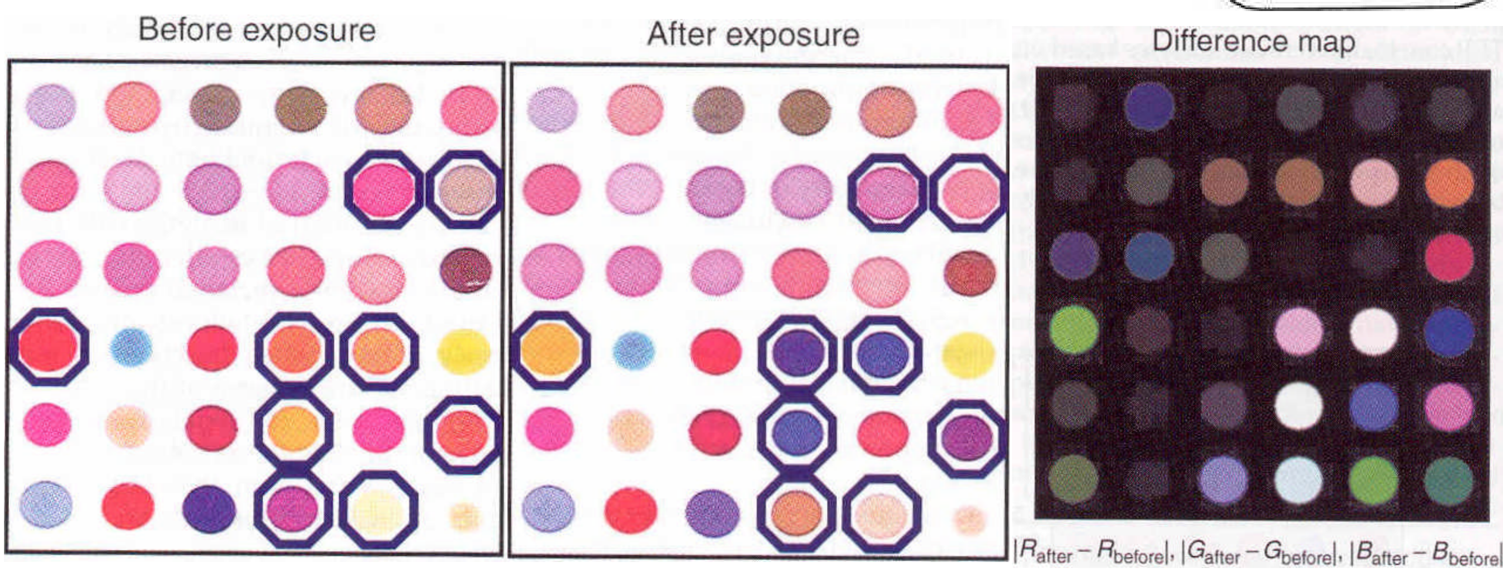

# Defense & Security




E-Nose sensor substrate, with 8 screen-printed Pd-Au electrode sets. Ryan et al, MRS Bull. 29 (2004) 714.




Electronic Tongues. Winquist et al, MRS Bull. 29 (2004) 726




Optoelectronic Nose by Colometric Sensor. Suslick, MRS Bull. 29 (2004) 720.

# Electronic Noses; Electronic Tongues

# The world has become a darker place in last 50 years

**Though the Sun remains as bright as ever, scientists believe less sunlight is reaching the Earth's surface because of pollution**

NEW YORK — In the second half of the 20th century, the world became, quite literally, a darker place.

Defying expectation and easy explanation, hundreds of instruments around the world recorded a drop in sunshine reaching the surface of Earth, as much as 10 per cent from the late 1950s to the early 1990s, or 2 to 3 per cent per decade.

In some areas like Asia, the United States and Europe, the drop was even steeper. Hong Kong, for instance, saw a 37 per cent decrease in its sunlight.

No one is predicting that it may soon be night all day, and some scientists believe the skies have brightened in the past decade as the suspected cause of global dimming — air pollution — clears up in many parts of the world.

Still, the dimming trend — noticed by a handful of scientists two decades ago, but dismissed at the time as unbelievable — is now attracting wide attention. Research on global dimming and its implications for weather, water supplies and agriculture, will be presented in Montreal next week at a joint meeting of the American and Canadian geological societies.

Dr James Hansen, director of Nasa's Goddard Institute for Space Studies in New York City, said scientists had long known that pollution particles reflected some sunlight, but added that now they are realising the magnitude of the effect.

"It's occurred over a long time period, so it's not something that perhaps jumps out at you as a person in the street," he said. "But it's a large effect."

Satellite readings show that the Sun remains as bright as ever, but less and less sunlight has been making it through the atmosphere to the ground.

Pollution dims sunlight in two ways, scientists believe. Some of the light bounces off soot particles in the air and back into outer space. Pollution also causes more water droplets to condense out of air, leading to thicker, darker clouds that also block more light.

For that reason, the dimming effect appears to be more pronounced on cloudy days than sunny ones. In some more pristine regions, there has been little or no dimming.

But the dynamics and effects of global dimming are not fully understood. Antarctica, which would be expected to have clean air, has also dimmed.

"In general, we don't really understand this thing that's going on," said Dr Shabtai Cohen, a scientist in Israel's agriculture ministry who has studied global dimming for a decade, "and we don't have the whole story".

The instrument to measure sunshine, called a radiometer, is simple in design: a black plate under a glass dome. Like asphalt in summer, the black plate turns hot as it absorbs the Sun's energy; its temperature tells the amount of sunlight that has shone on it.

Since the 1950s, hundreds of radiometers have been installed, from the Arctic to Antarctica, dutifully recording sunshine levels.

Not every scientist is convinced that the dimming has been pronounced. While radiometers are simple instruments, they do require periodic calibration and care.

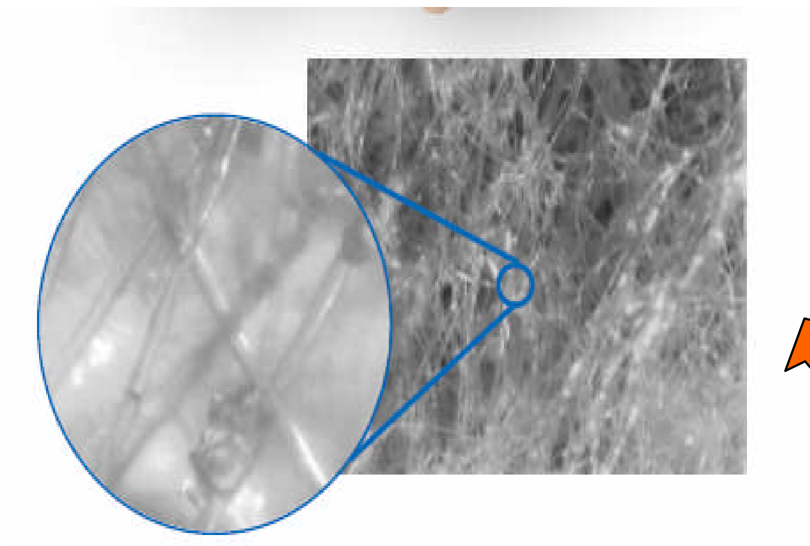
Dirt on the glass dome will block light, leading to erroneous indications of declining sunlight. Also, all the radiometers have been on land, leaving the effect over water, which occupies three-quarters of the Earth's surface, to supposition.

"I see some datasets that are consistent and some that aren't," said Dr Ellsworth Dutton, chief of the radiation monitoring group at the National Oceanic and Atmospheric Administration.

"Certainly, the magnitude of the phenomenon is in considerable question."

— THE NEW YORK TIMES

Source: The Straits Times, 14th May 2004, Page H16.




*(40x magnification shows the spun fiberglass skin of the filter pad.)*

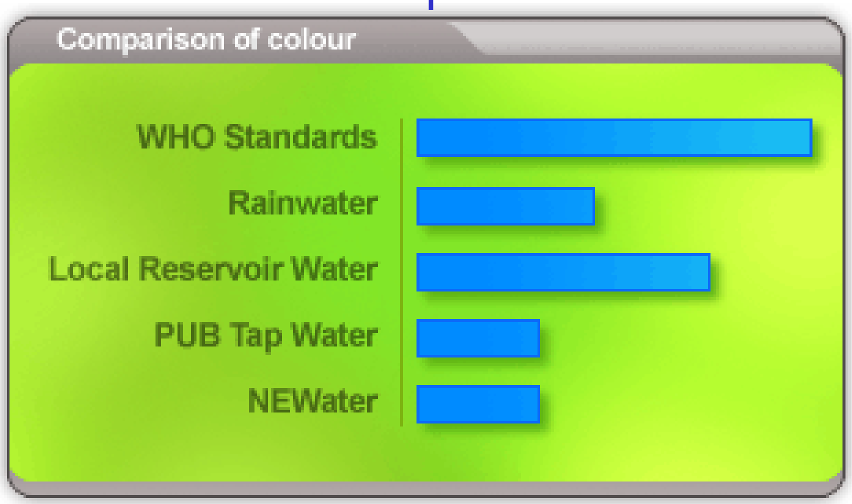
## Air Filter Membranes

# Environmental Engineering & Biotechnology

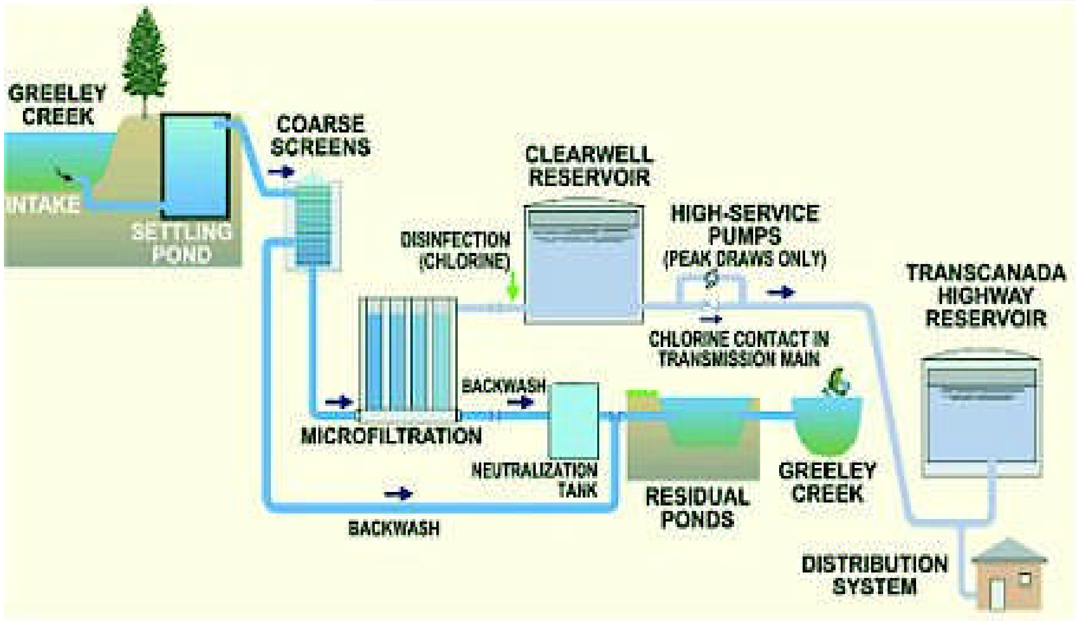
NUS National University of Singapore

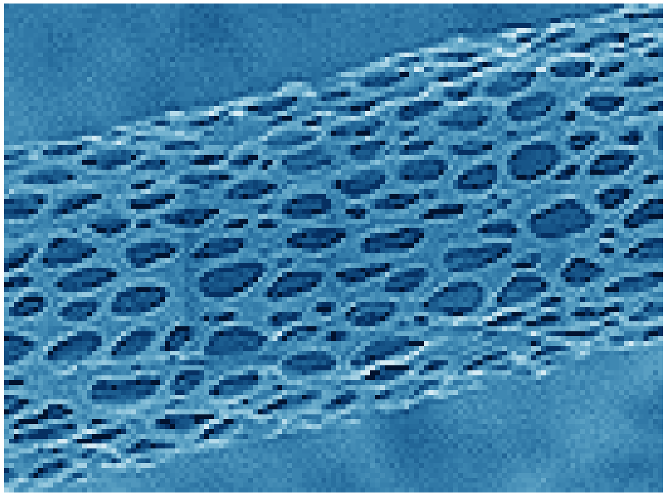

## Water


### Standards of potable water

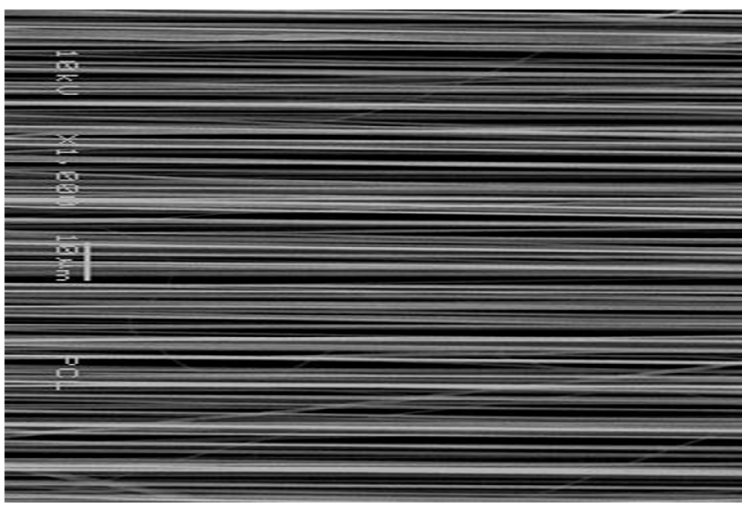



http://www.pub.gov.sg/NEWater





Water purification via membrane filters



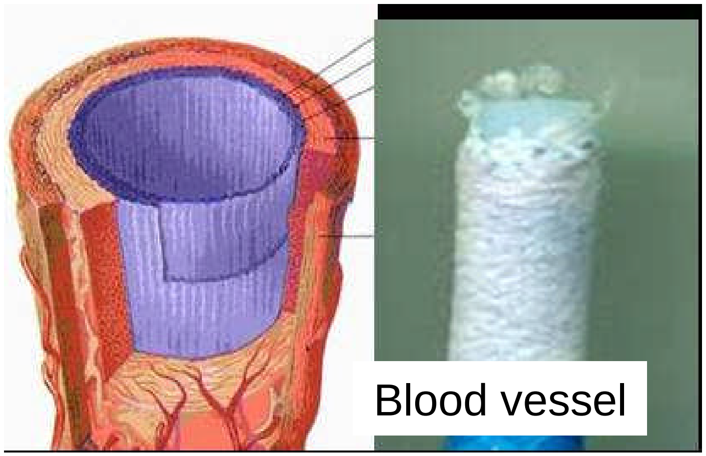
## Liquid Filter Membranes

# Bioengineering

## Anti-microbial Clothing

# Bioengineering

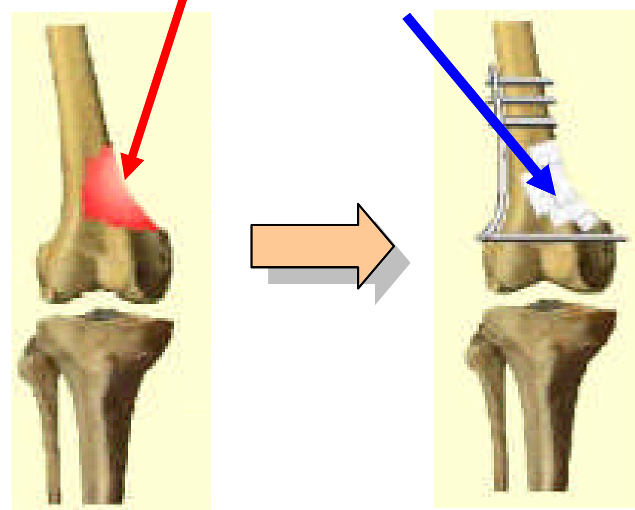
NUS
National University of Singapore



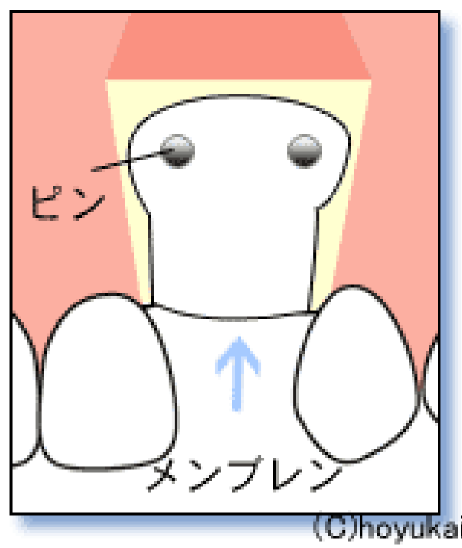
Blood vessel

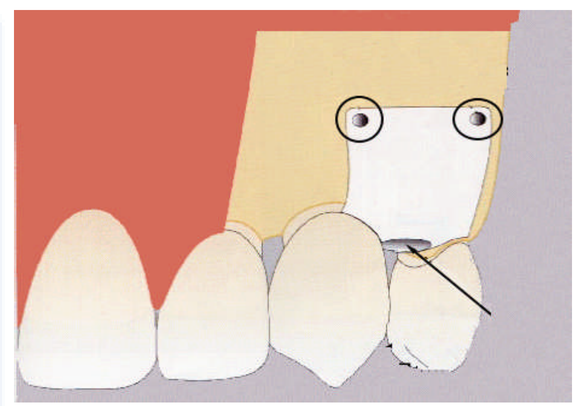
Wound dressing
www.hospitalmanagement.net

## Bone Regeneration



Uemura et al, Biomaterials 24 (2003) 2277.

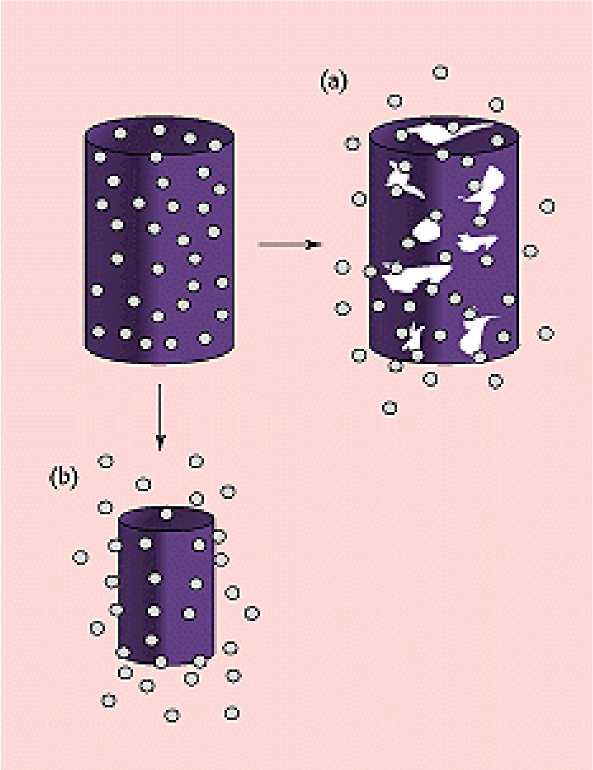


Peridontal Membrane

# Bioengineering

## Drug Delivery    Nano-Syringe

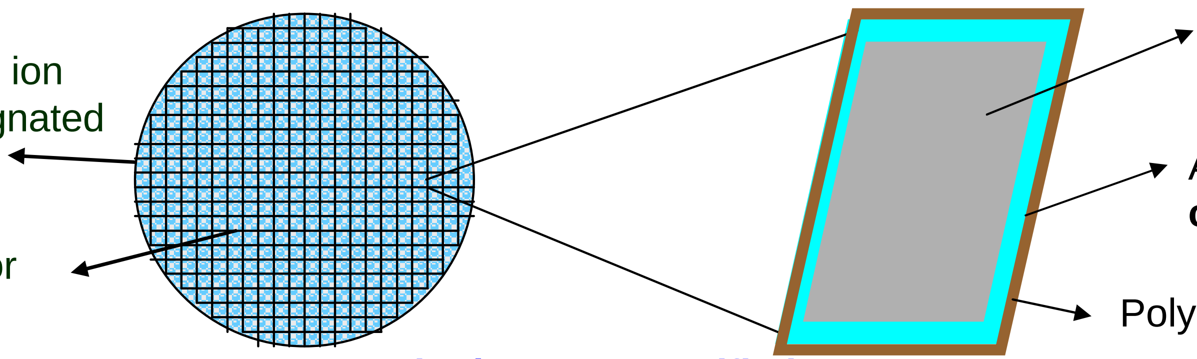



Ref. http://resurgence.gn.apc.org/issues/broadhead221.htm

# Bioengineering

National University of Singapore

## A novel patch for delivery of multiple drug types at controlled release rates




A single pore magnified

- The **hydrophilic drug** (water soluble antioxidant drugs, Vitamin C and analgesics) is coated onto the polymer surface by immersion and evaporation of water from drug solution
- The **hydrophobic drug** molecules (steroid hormones, vitamins A and E) are dissolved with the **fatty acid** and impregnated on the surface of the nanofiber mesh

| Company | Product | Constituent drug | Comments |
|---|---|---|---|
| Alza | D-Trans, E-trans | Nicotine patches | Min 1 day, max 1 week |
| 3M | Minitran and Climara | Nitroglycerin, Estradiol | For heart attacks<br>Max. 1 day |
| Genetronics | Medpulser, Wave pulser | Proteins | Electroporation technique |
| Cilag-Janssen | Duragesic | Fenantyl | Narcotics, for pain management<br>10 day patches available |
| Under Investigation | Activetex | Wound healing, anti bacterial | Available only for water soluble drugs |

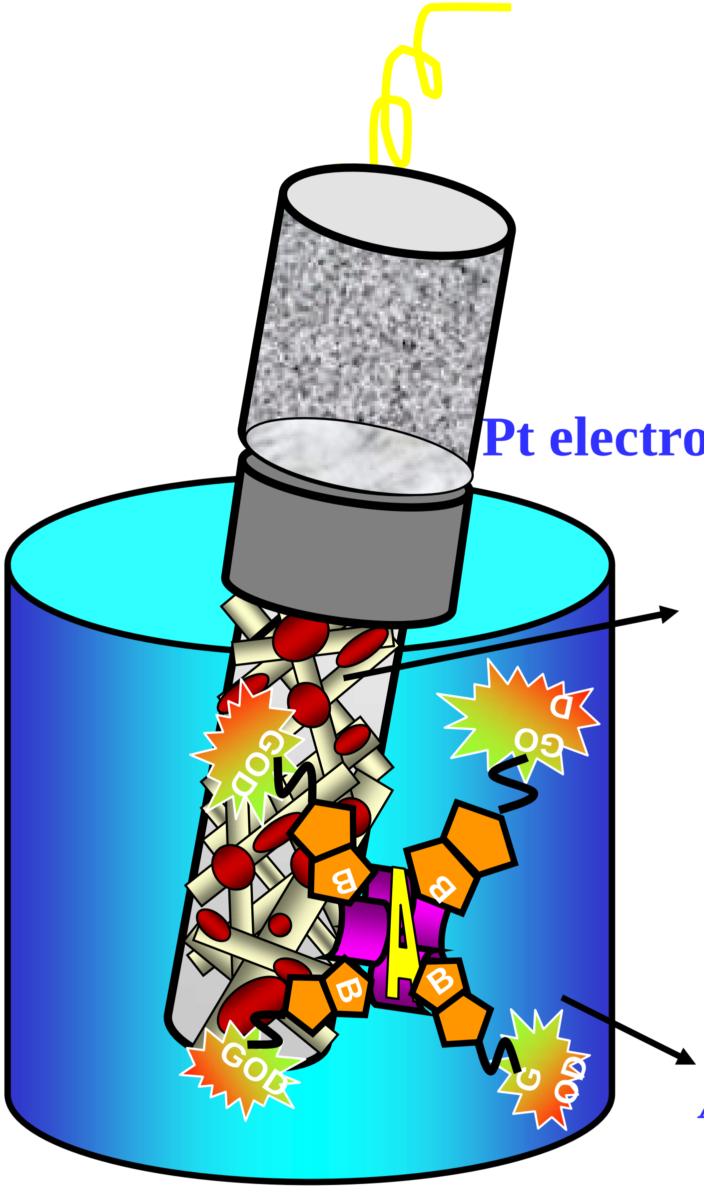
Commercial products

*ICNT, Nov 7-11, 2004*

# Bioengineering

NUS National University of Singapore

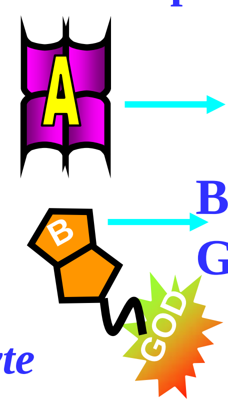
## Glucose Sensors




Pt electrode

Nylon-6 nanofiber immobilized with Au nanoparticles

Analyte




Avidin

Biotinylated Glucose Oxidase

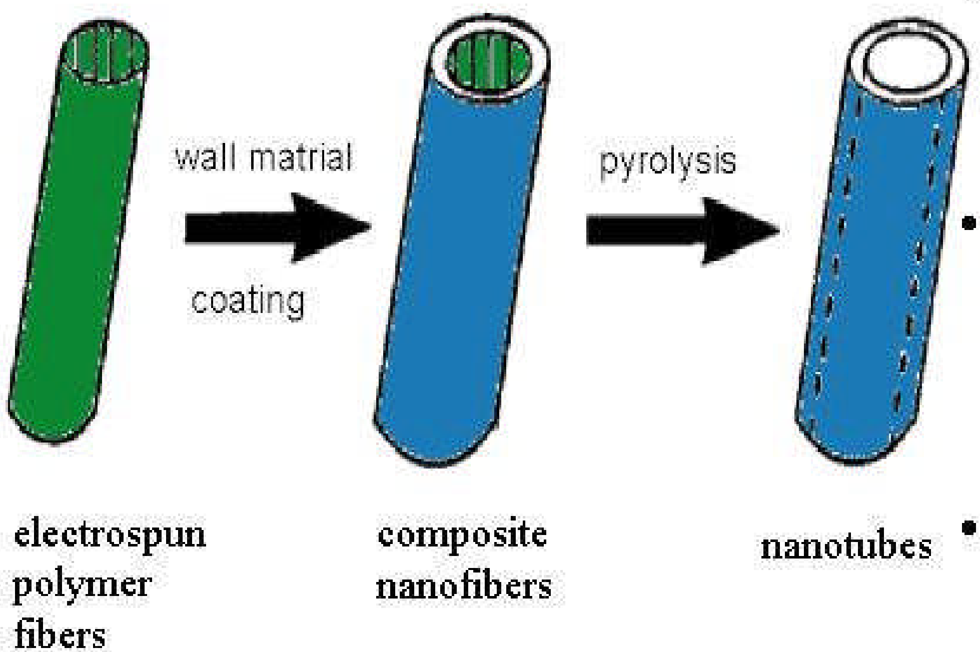
### Commercial Technologies


| *Name and Company* | *Type / Technology* | *Features* |
|---|---|---|
| Active meter (Accu-Chek) | Incision | Response time: 5s<br>Clarke electrode ampereometric sensors |
| Freestyle (Therasense) | Incision | Coulometric sensors |
| Elite Active Strip (Bayer-Ascensia) | Reflectance colorimetry | Not economically viable for frequent glucose monitoring |
| One touch Ultra (Lifescan) | Incision | Response time : 5s<br>has interference if uric acid is present in the sample |

### Goals

- Higher Sensitivity (10 -9– 10-12 M )
- Higher Specificity

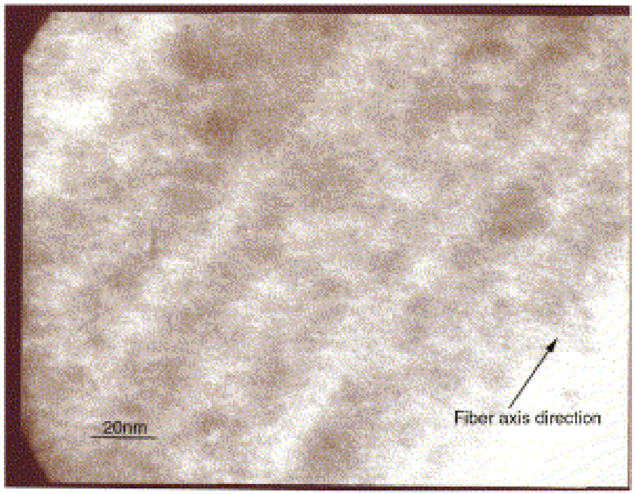
# Thermally Conducting Nanotubes and Fibers -How?



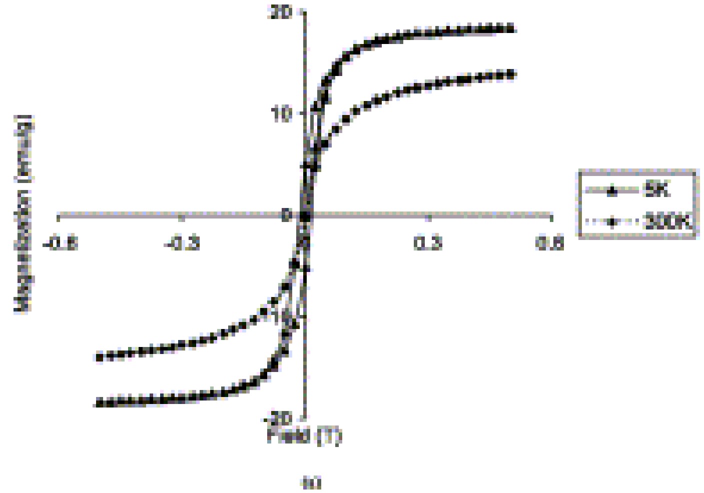

- produce degradable polymer fiber template by electrospinning
- coat fibers with desired wall materials using various types of deposition techniques
- selectively remove core material by thermal degradation

M. Bognitzki, A. Greiner and etc., Adv. Mater. 2000, 12, No.9, 637-640

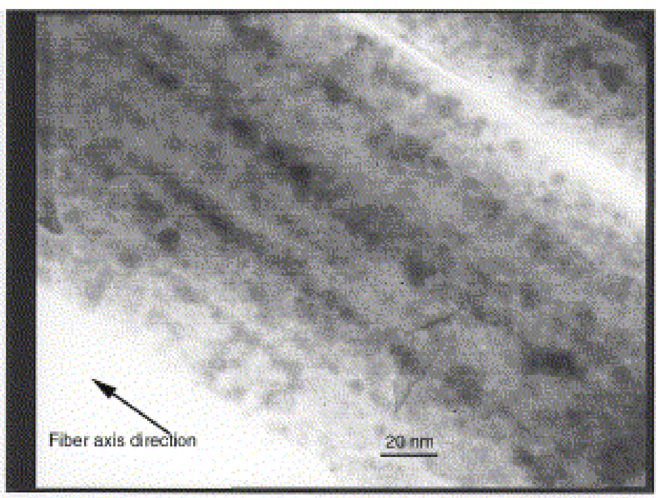
# Energy & Electronics



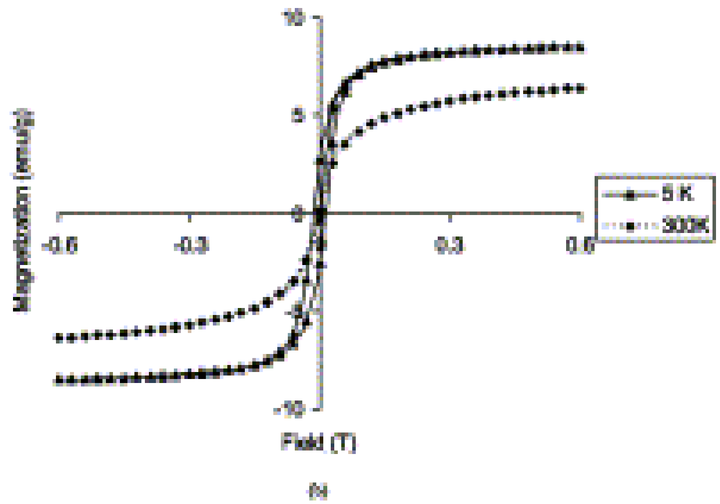
(a)




Poly (ethylene oxide) nanofiber with 28 wt% magnetite nanoparticles



(b)




Poly (vinyl alchohol) nanofiber with 8 wt% magnetite nanoparticles

TEM images of superparamagnetic nanofibers

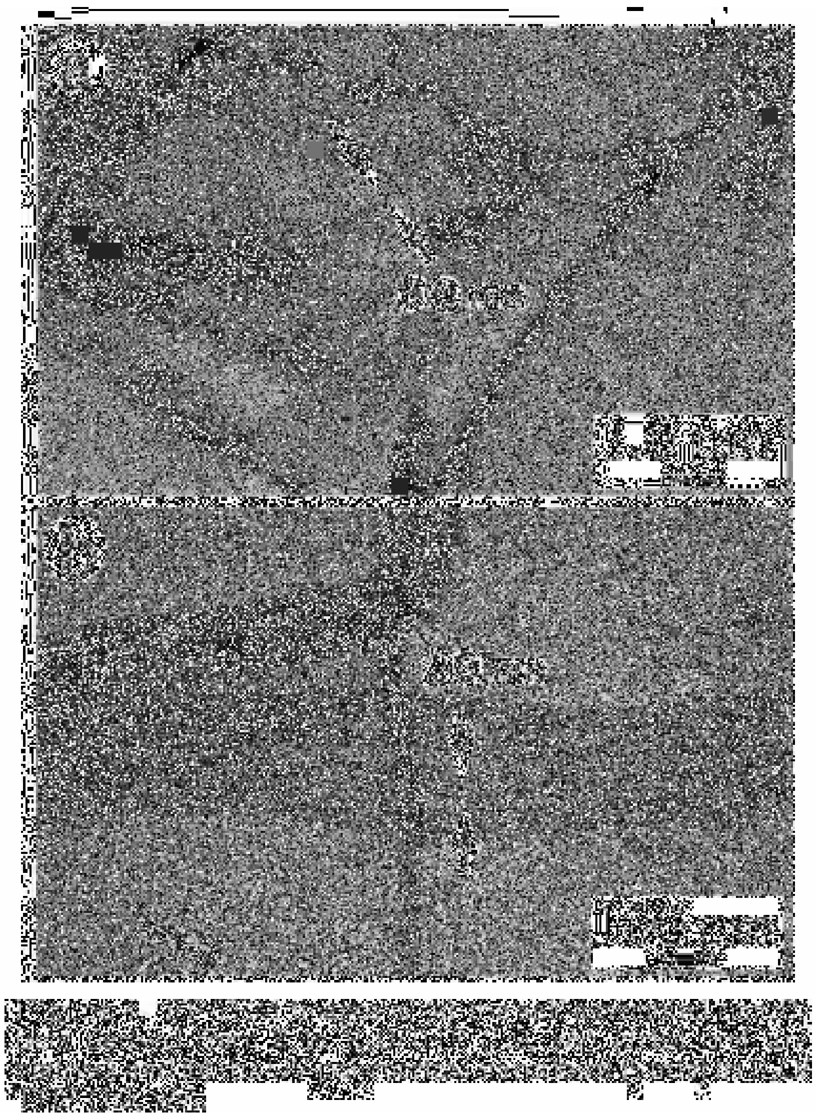
Magnetization curves of supermagnetic nanofibers

# Outline

NUS National University of Singapore

- Motivation for Nanofibers
- Quest for Polymer Nanofibers
- Electrospinning - State of the Art
- Affinity Membranes
- Tissue Engineering

# Self-Assembly



Matsuda et al., Macromolecules, 1996

## Polymers to have been assembled into nanofibers

- **PS-b-PCEMA**
- **PS-b-PCEMA-b-PtBA**
- **Peptide-Amphiphile**
- **Polyphenylene Dendrimer**
- **Bolaform glucosamide**

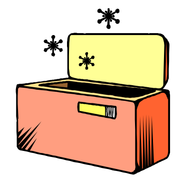
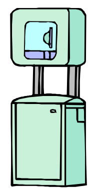
## Specific information

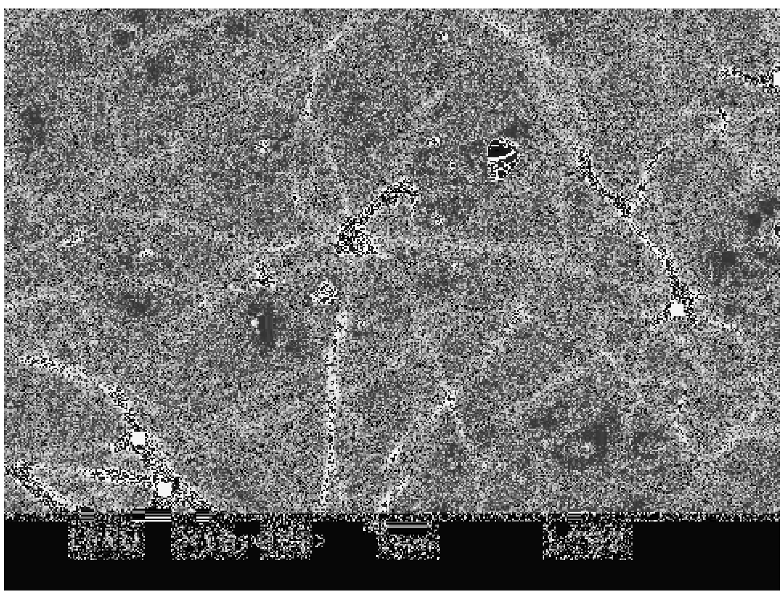
- **Fiber diameter: 7 – 100 nm**
- **Fiber length: 1 – several micron**

# Phase Separation



## Method

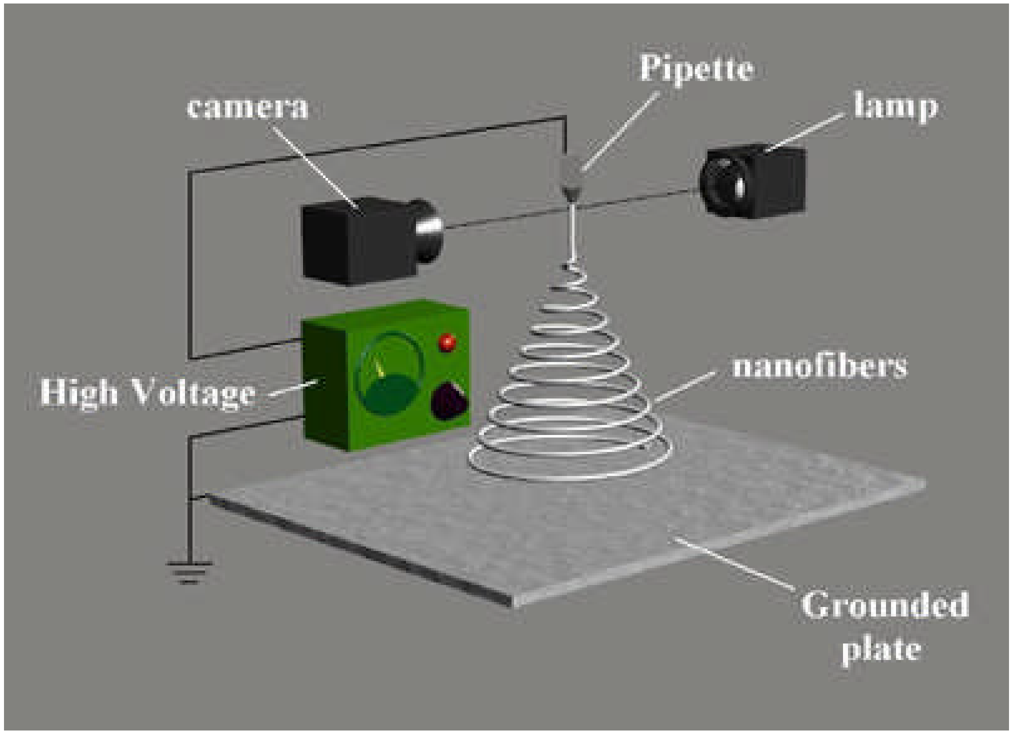
- Polymer dissolution 
- Phase separation and gelation 
- Solvent exchange 
- Freeze-drying 



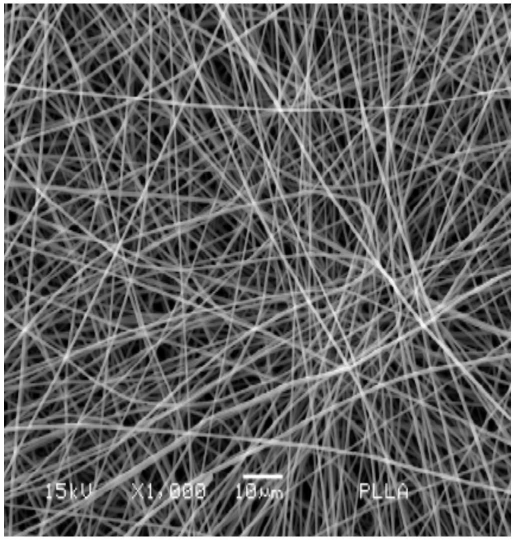
## Specific Information

- No special equipment requirement
- Simple procedure
- Fibers in nano scale (50 -500 nm)
- Shape is determined by the mold

# Electrospinning




*(http://fluid.ippt.gov.pl/sblonski/nanofibres.html)*




**Random Mats**

# Comparison of Various Methods

NUS National University of Singapore

| Process | Laboratory or industrial process | Ease of processing | Advantages | Limitations |
|---|---|---|---|---|
| **Self-Assembly** | Lab | Difficult | | • Complex process.<br>• Limited to a few polymers. |
| **Phase Separation** | Lab | Easy | • Simple process.<br>• Batch-to-batch consistency is easily achieved.<br>• Mechanical properties of the mat can be tailored by adjusting polymer concentration. | • Limited to a few polymers. |
| **Electro-spinning** | Lab;<br>Potential for industrial processing | Moderately easy | • Simple process.<br>• Cost effective.<br>• A large variety of polymers can be electrospun.<br>• Long, continuous nanofibers can be produced.<br>• Production of aligned nanofibers is feasible.<br>• Potential to combine with secondary proecss (ex. textile technology) | |

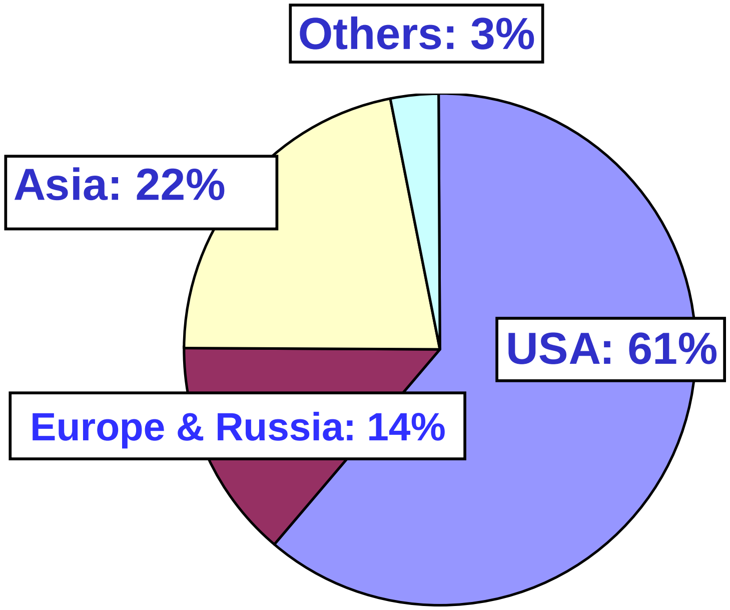
# Outline

- Motivation for Nanofibers
- Quest for Polymer Nanofibers
- **Electrospinning - State of the Art**
- Affinity Membranes
- Tissue Engineering

# Snapshot of Electrospinning Worldwide

## Worldwide more than 70 research groups are working on electrospinning

### Universities & Institutes

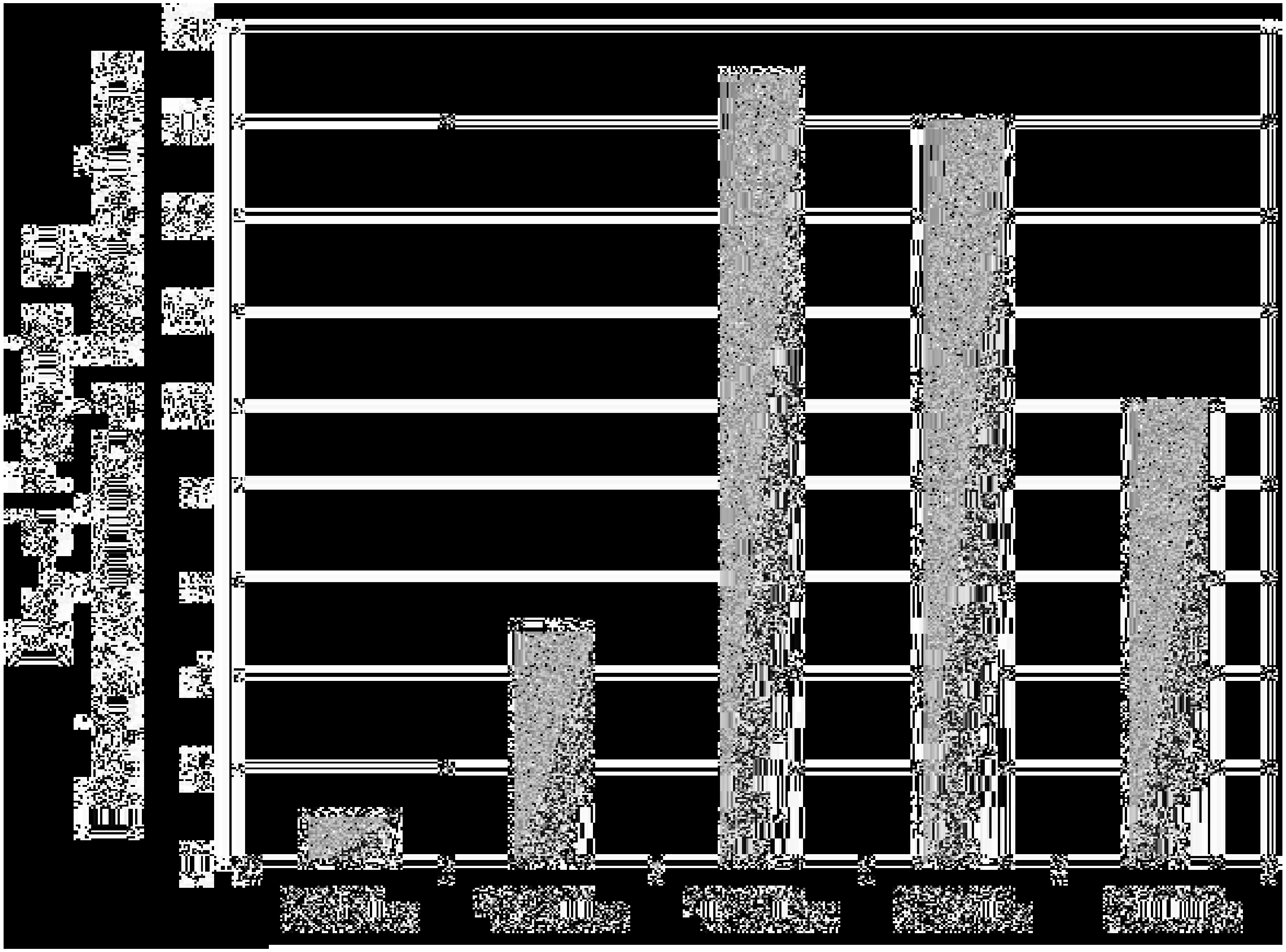



### Industry

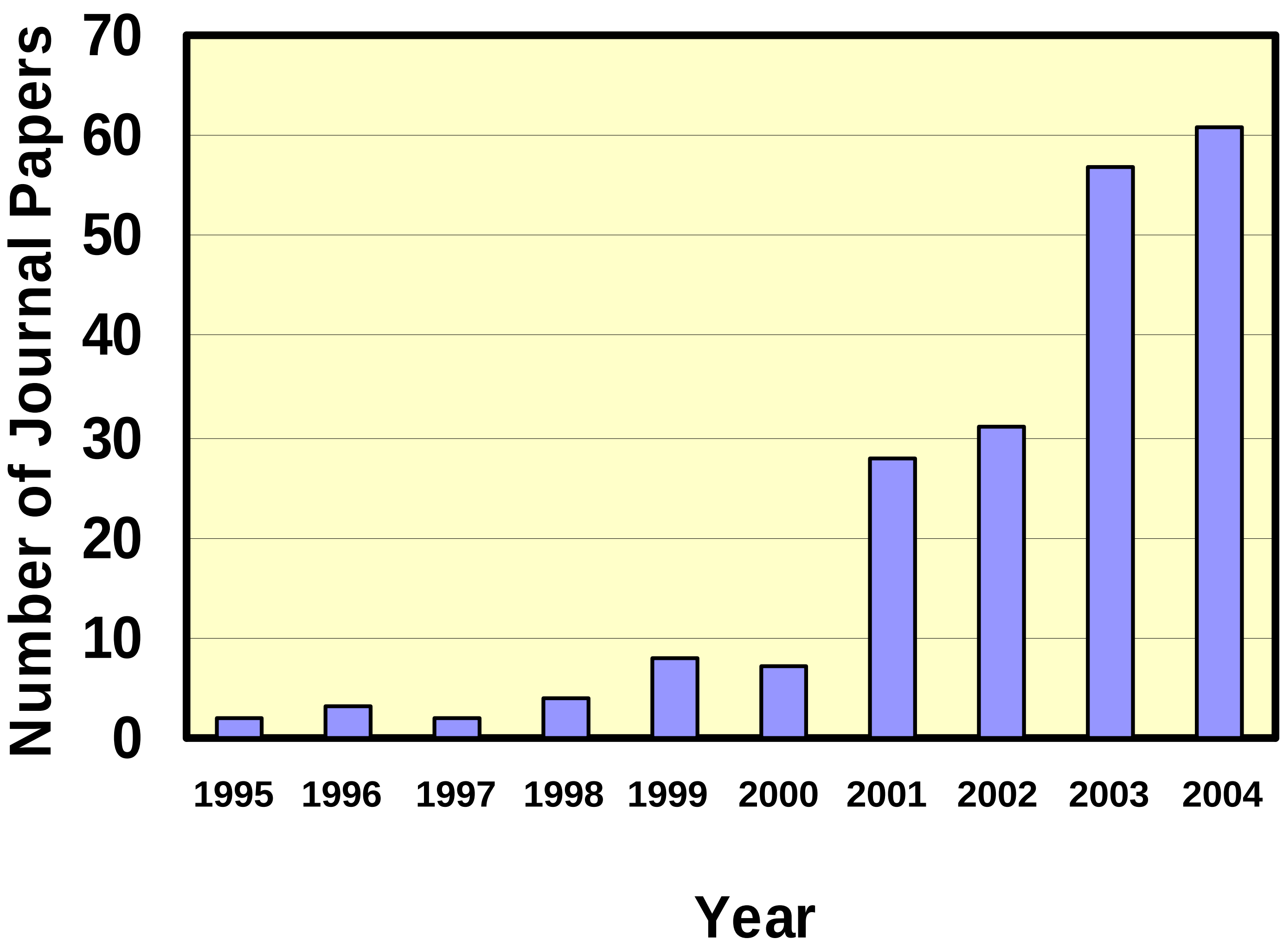
- Donaldson Filtration Solutions, USA
- Ahlstrom, USA
- Parker Filtration, USA
- Fleetguard, USA
- Hollingsworth & Vose Company, USA
- eSpin Technologies, USA
- Donaldoson Company, USA
- Hollingsworth & Vose GMBH & CO, Germany
- Tejin, Japan

**Strategic Research Group for Nanofiber Technology (Japan Society of Fiber Science and Technology)**

# US Patents on Electrospinning



# Papers on Electrospinning



# Research Areas

NUS National University of Singapore

## Defense & Security

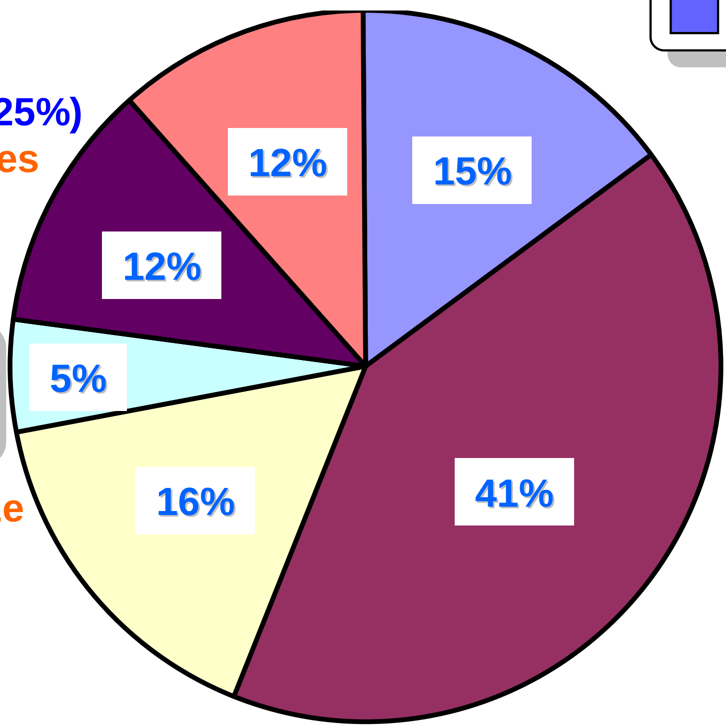
- Composite Reinforcement (35%)
- Biosensor (17%)
- Chemical Sensor (17%), Optical Sensor (17%)
- Gas Sensor (13%)

## Energy & Electronics

- Catalyst (35%)
- Battery/Fuel Cells (22%)
- Electrode (17%)
- Capacitor, Semiconductor (25%) FET, & Optoelectronic Devices

## Processing

- Jet Formation (50%) (Experimental)
- Jet Formation (20%) (Modeling)
- Spinning Setup (30%)




## Environmental Eng. & Biotechnology

- Stop & Kill, and Stop & Neutralize Filter Membranes (100%)

## Characterization

- Morphology (39%)
- Structure (31%)
- Chemical (18%)
- Mechanical (12%)

## Bioengineering

- Tissue Engineering & Wound dressing (81%)
- Drug Delivery (19%)

# Spinnable Combinations
## - Polymer Nanofibers -

NUS National University of Singapore

| Category | Polymers | Solvent |
|---|---|---|
| Thermoplastic polymers | Polystyrene, PS | tetrahydrofuran, dimethylformamide, toluene, chloroform |
| | Polycarbonate, PC | dichlormethane, chloroform, tetrahydrofuran, |
| | Polymethacrylate, PMMA | tetrahydrofuran, acetone, chloroform |
| | Polyvinylchloride, PVC | mixture of tetrahydrofuran & dimethylformamide |
| | Polyethylene Terephtalate, PET | mixture of dichloromethane & trifluoroacetic acid |
| | Nylon6,6, PA-6 | formic acid |
| | nylon-4,6, PA-4,6 | formic acid |
| | Polyamide, PA | dimethylacetamide |
| Biocompatible Biodegradable polymers | Polyurethanes, PU | dimethyl formamide |
| | Polyvinyl alcohol, PVA | distilled water |
| | polylactic acid, PLA | dimethyl formamide, dichlormethane |
| | Polycaprolactone, PCL | chloroform, toluene, dichloromethane |
| | Polyethylene gricol, PEG | chloroform |
| | Polylactide-co-glycolide, PLGA | mixture of tetrahydrofuran & dimethylformamide |
| | polyethylene-co-vinyl acetate, PEVA | |
| | polyethylene-co-vinyl alcohol, PEVOH | mixture of Isopropanol & water |
| | Polyethylene oxide, PEO | distilled water, chloroform, acetone |
| | Collagen | hexafluoro-2-propanol |

# Spinnable Combinations
## - Polymer Blend/Composite Fibers -

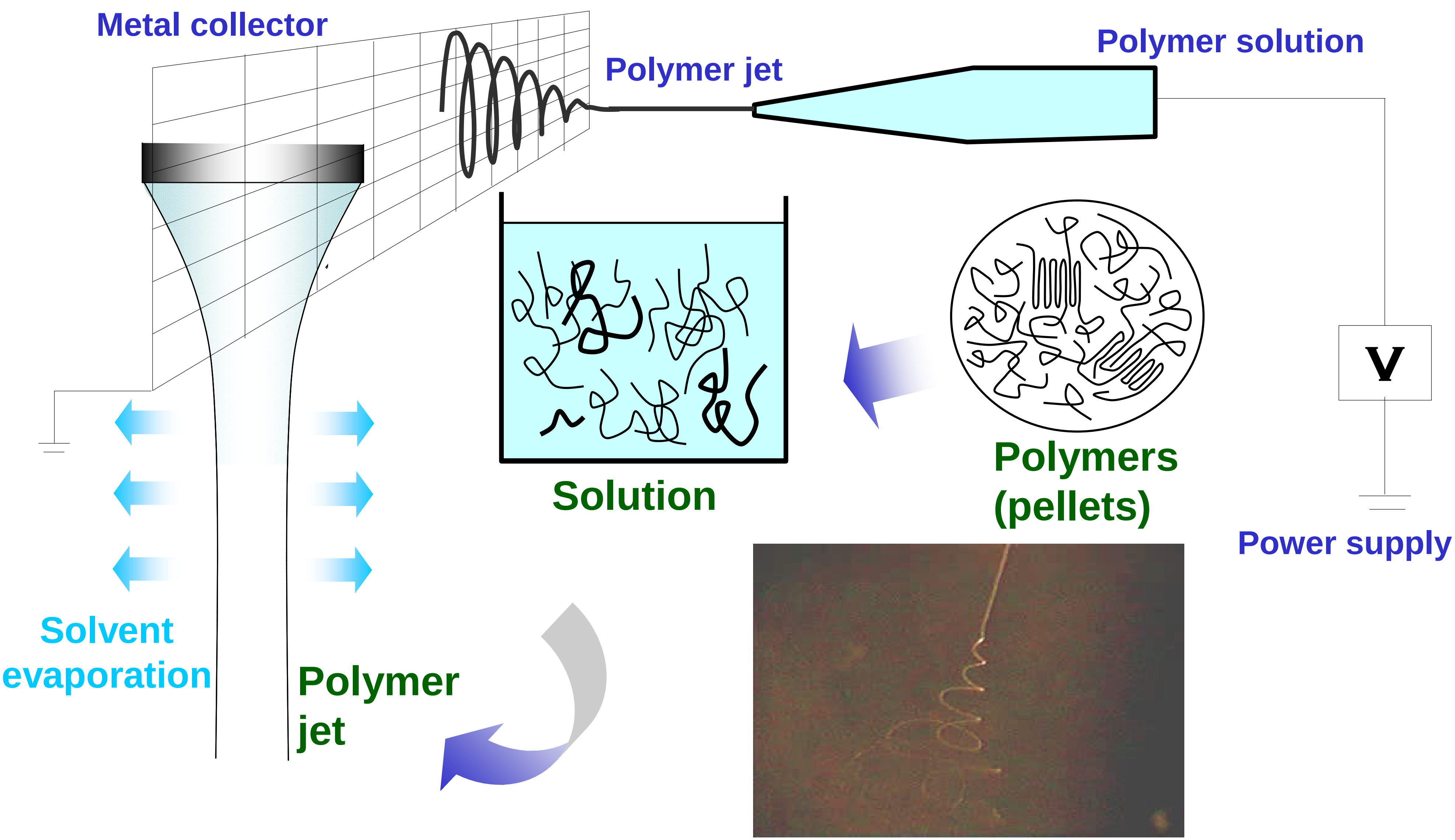
| Category | Polymers | Solvent |
|---|---|---|
| Polymer blends | PEVA/PLA | - |
| | PMMA / TAN | mixture of dimethyl formamide & toluene |
| | Polyaniline/PEO | chloroform, camphorsulfonic acid |
| | Collagen/PEO | Hydrochloric acid |
| | Silk/PEO | silk aqueous solutions |
| | Polyaniline/PS | chloroform, camphorsulfonic acid |
| | Fibrous calf thymus Na-DNA | mixture of water & ethanol |
| Composites (organic-inorganic hybrids) | Poly(vinil alcohol)PVA/Silica | distilled water |
| | Nylon6/montmorillonite (Mt) | hexa-fluoro-isopropanol (HFIP), mixture of HFIP & dimethylformamide |
| | Polyacrylnitrile (PAN) / TiO2 | - |
| | Polycaprolactone / gold or ZnO | - |
| | PCL/$CaCO_3$ | Chloroform |
| | PCL/carbon nanotube | Water |

# Electrospinning- Key Issues

NUS National University of Singapore

- How to produce nanofibers with controlled and uniform dimensions?
- How to place them in desired patterns and assemblies reproducibly?
- How to scale-up the process cost effectively?
- How to measure, predict, tailor and control physical, mechanical, chemical, biological, optical and electrical properties?

# Electrospinning Process



# Electrospinning Process Variables

National University of Singapore

## Solution properties

- Viscosity
- Polymer Concentration
- Molecular Weight of Polymer
- Electrical Conductivity
- Elasticity
- Surface tension

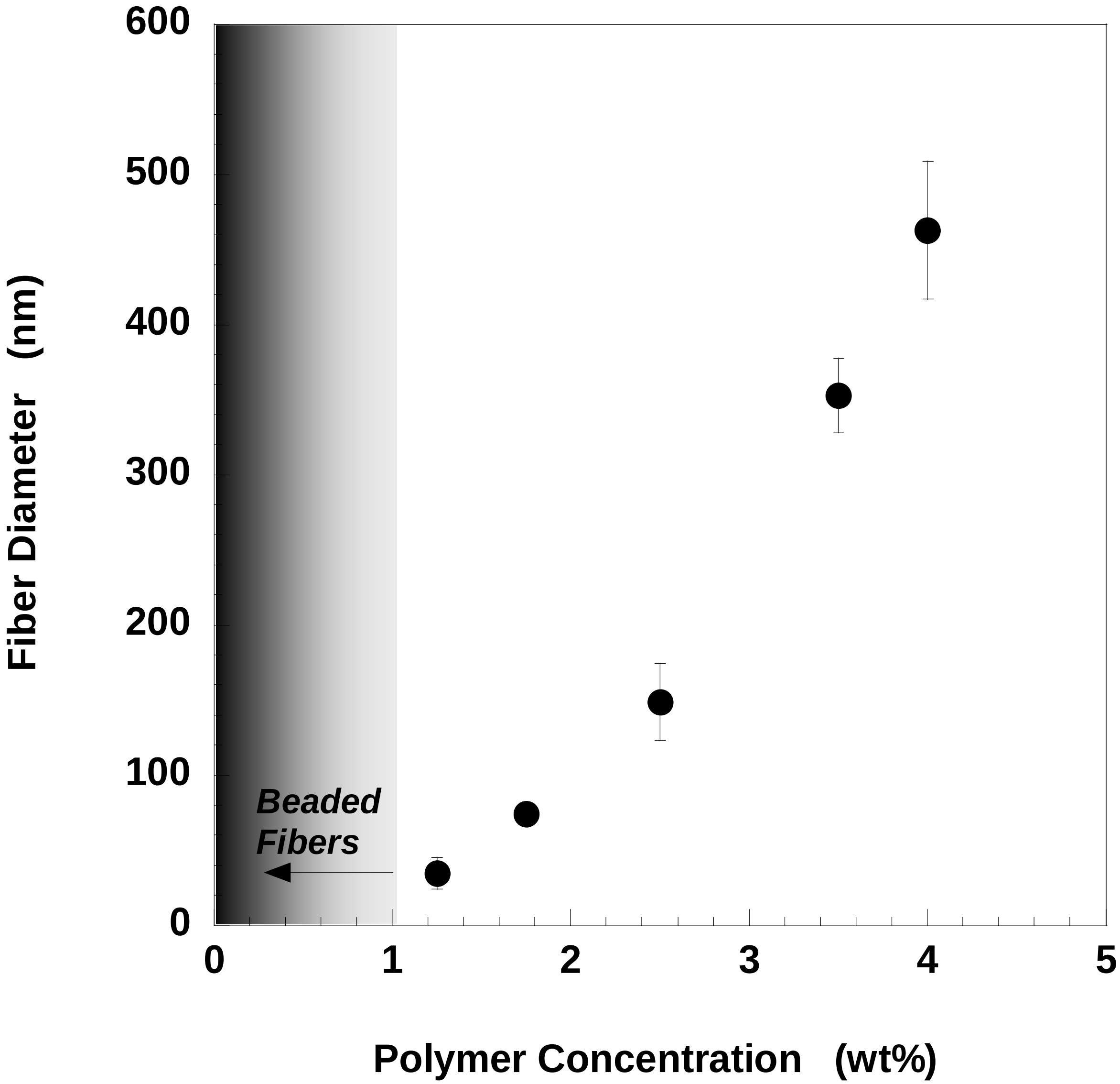
## Processing conditions

- Applied Voltage
- Volume Feed Rate
- Distance between Needle & Collector
- Needle Diameter
- Take-up Rate

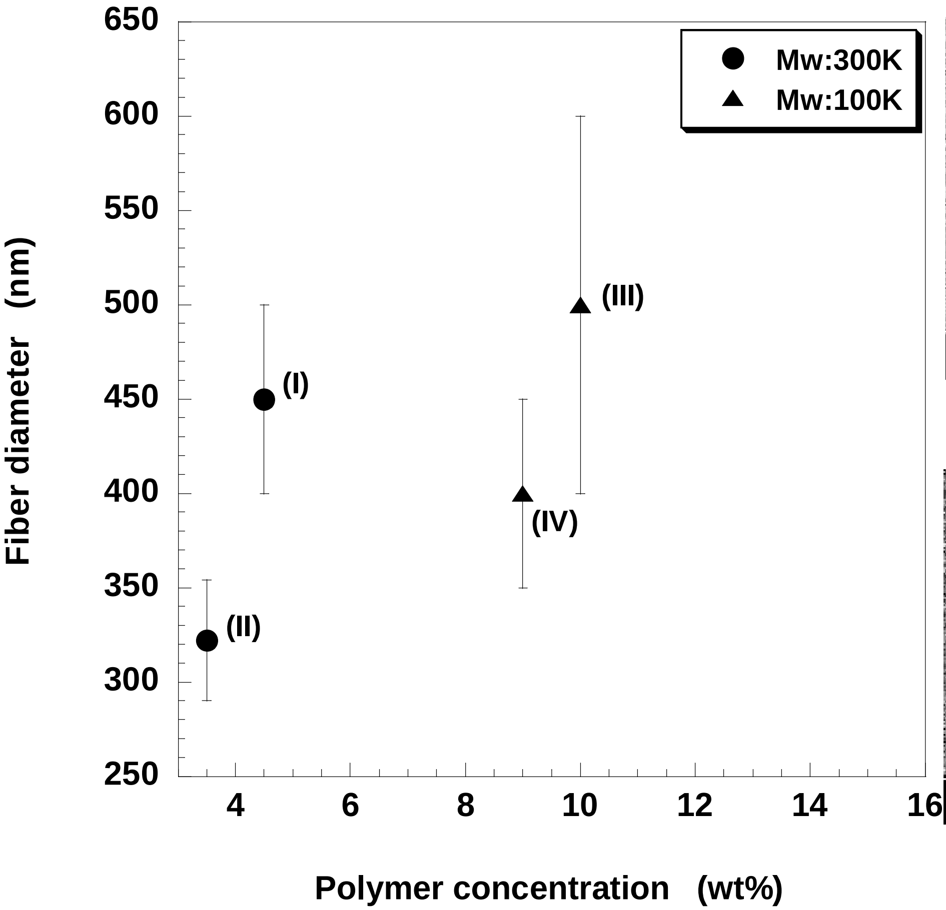
## Ambient conditions

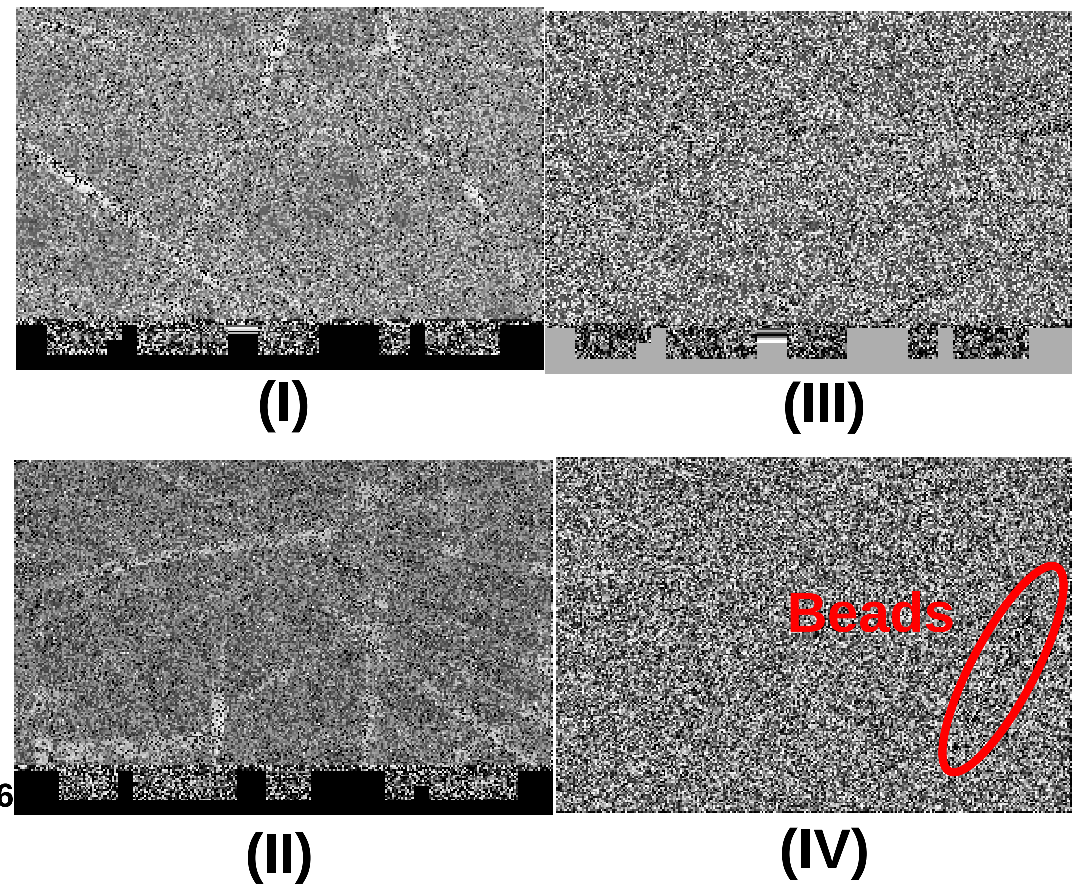
- Temperature
- Humidity
- Atmospheric Pressure

# Polymer concentration effects

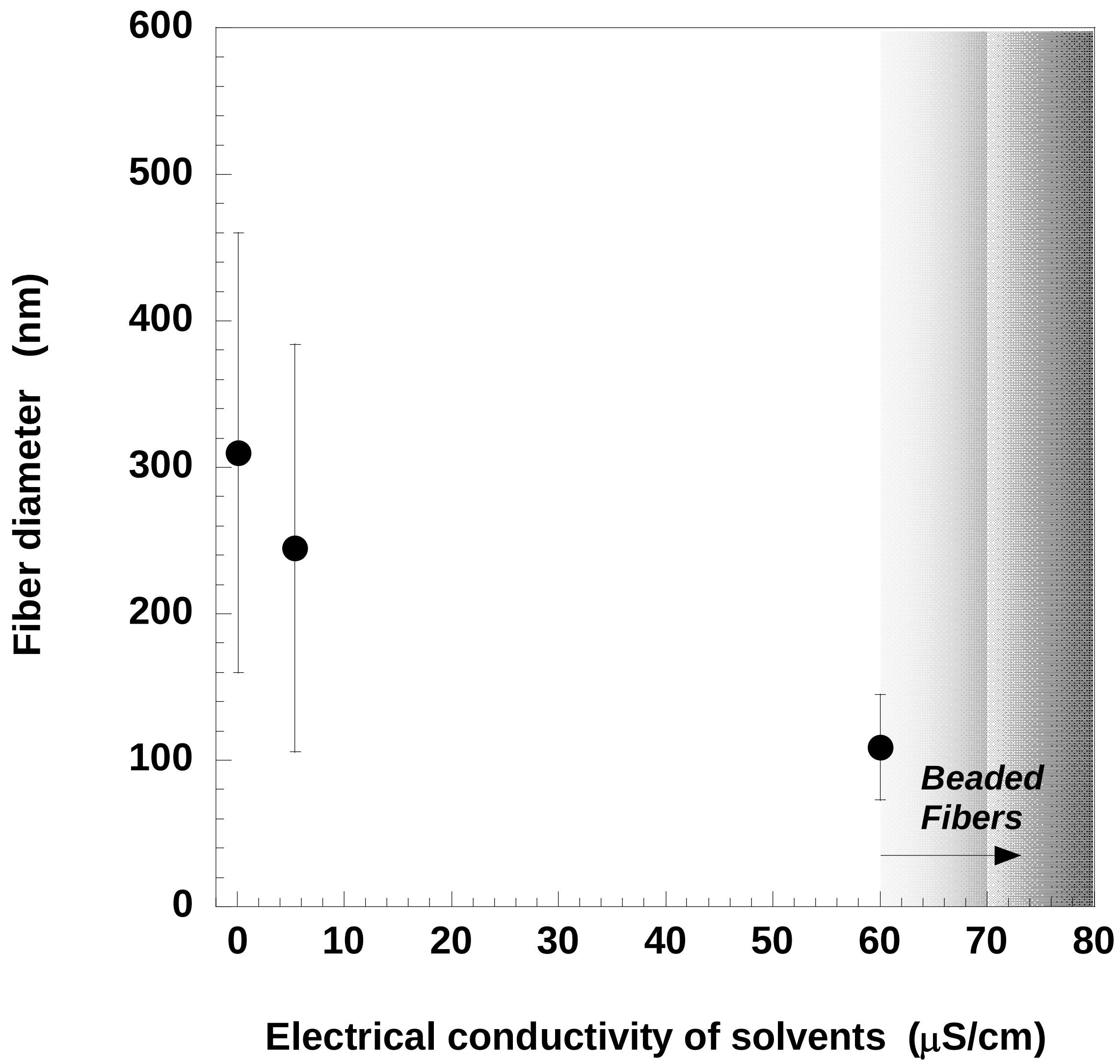


# *Molecular weight effects*

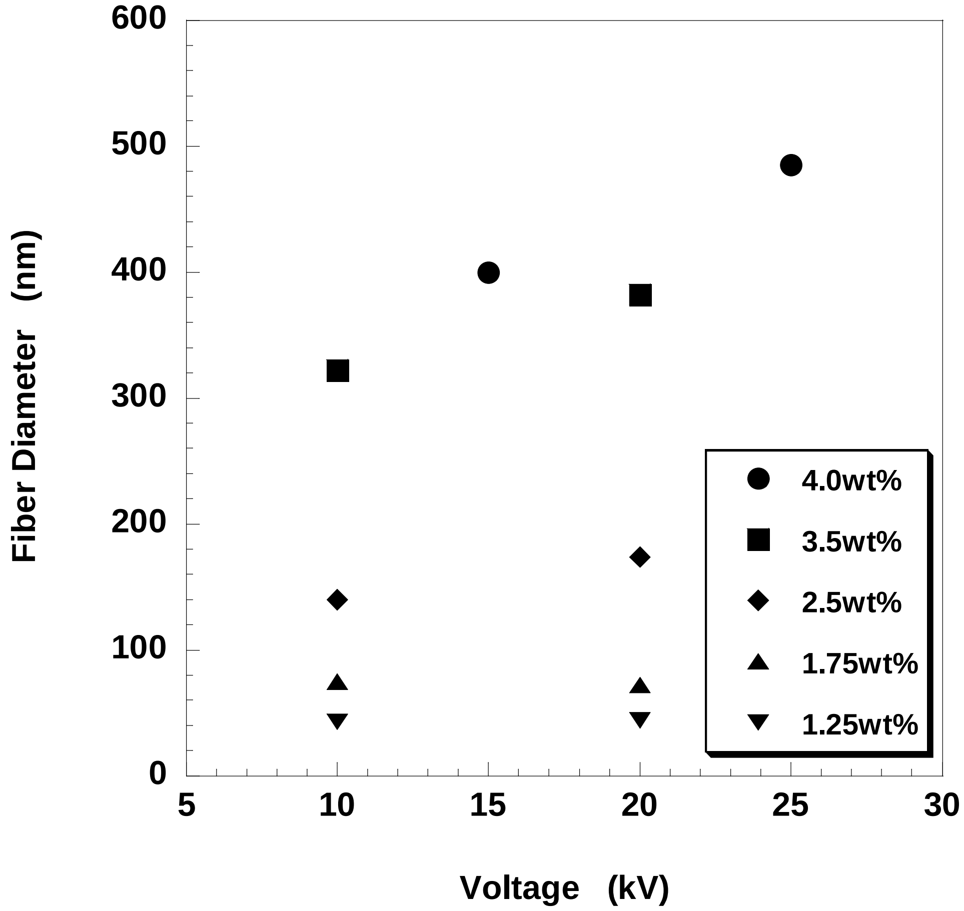


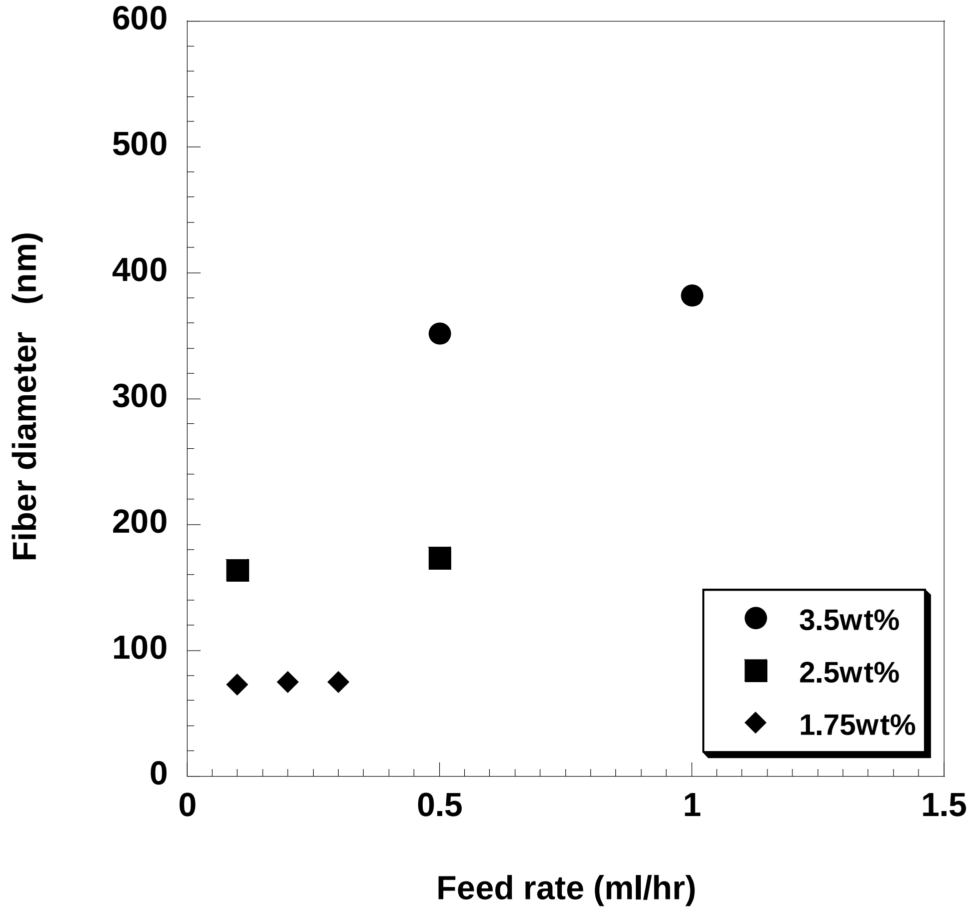



# Electrical conductivity effects

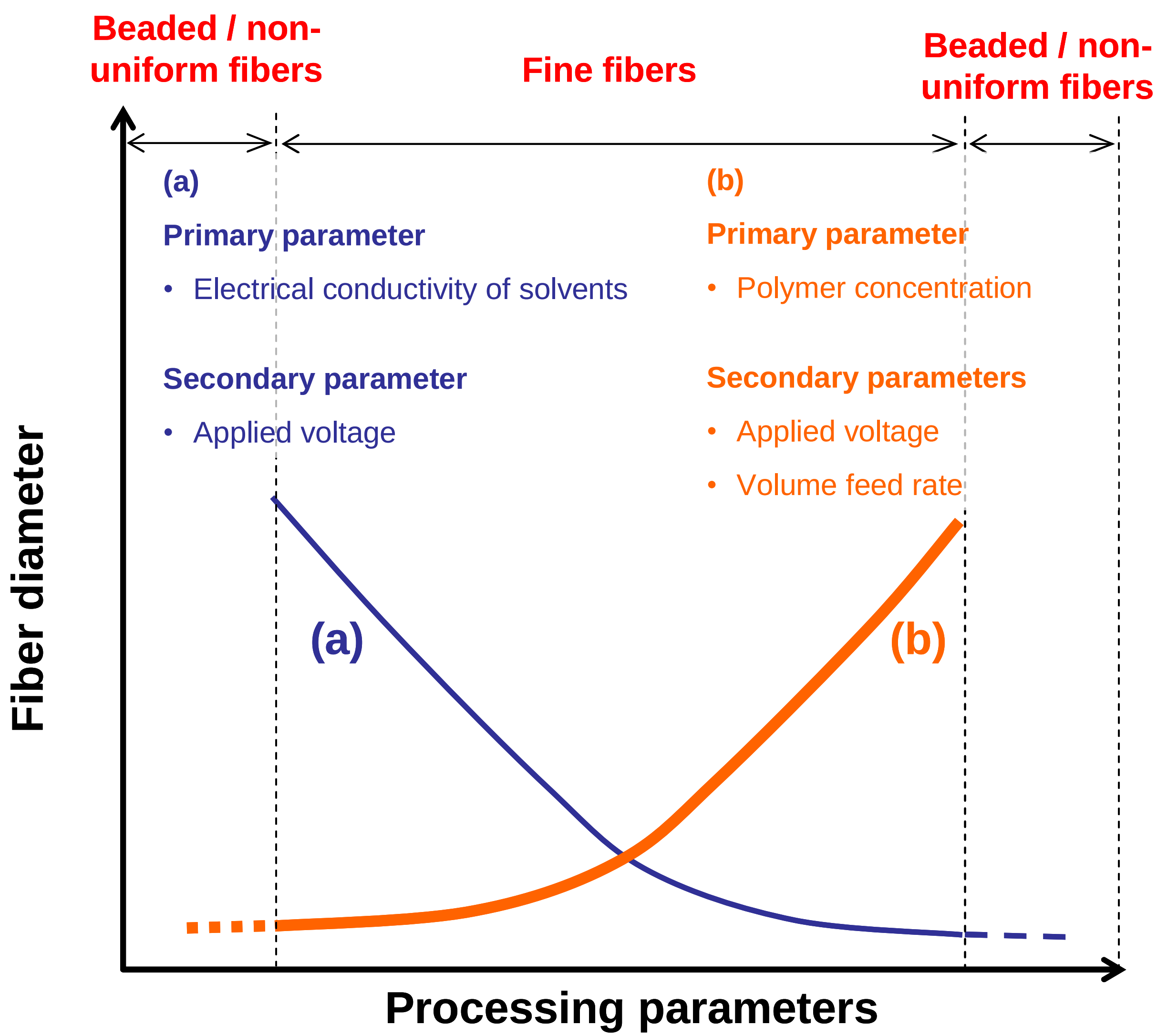


# *Voltage & Feed rate effects*



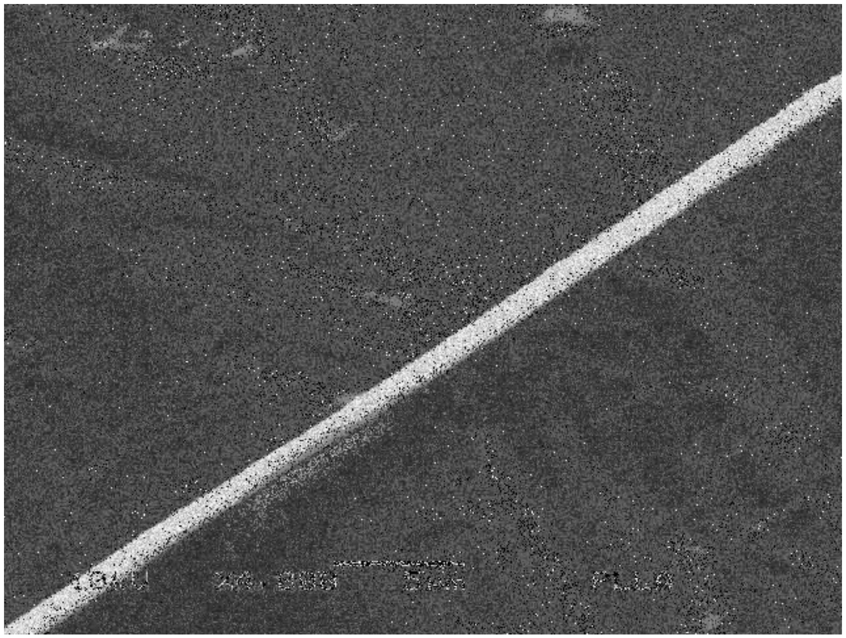



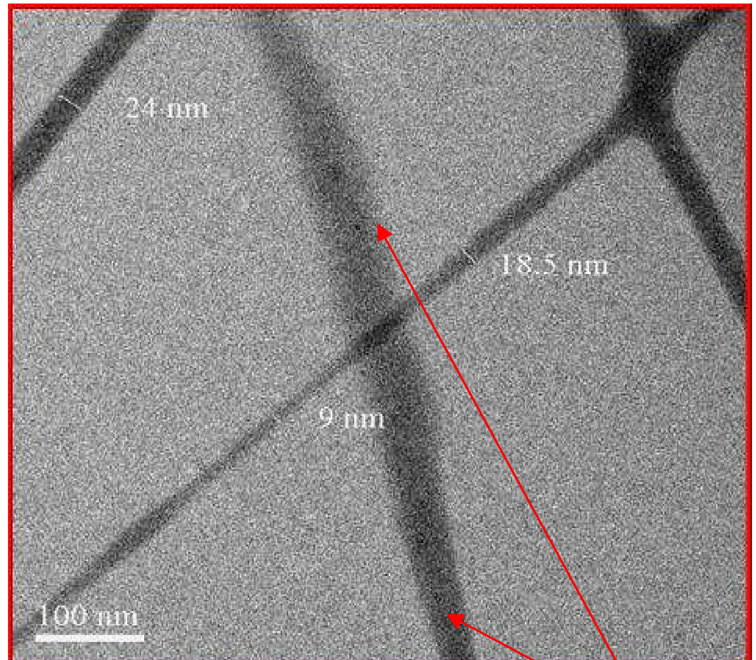
# Processing map



# Control Over Fiber Uniformity and Fineness?

NUS National University of Singapore






**Uniform Nanofiber**

**Non-uniform Fine Nanofiber**

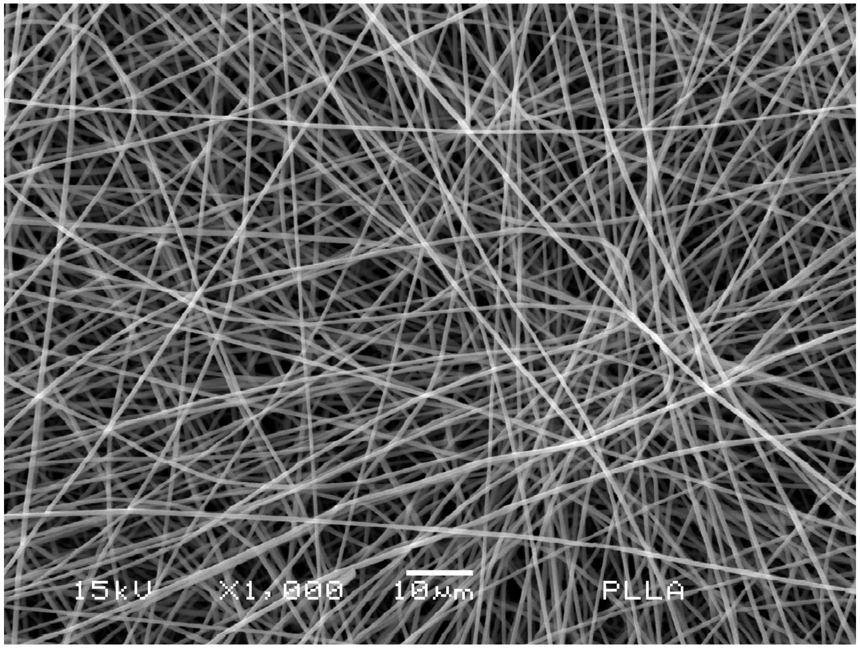
Seeram et al. Polymer, 2004

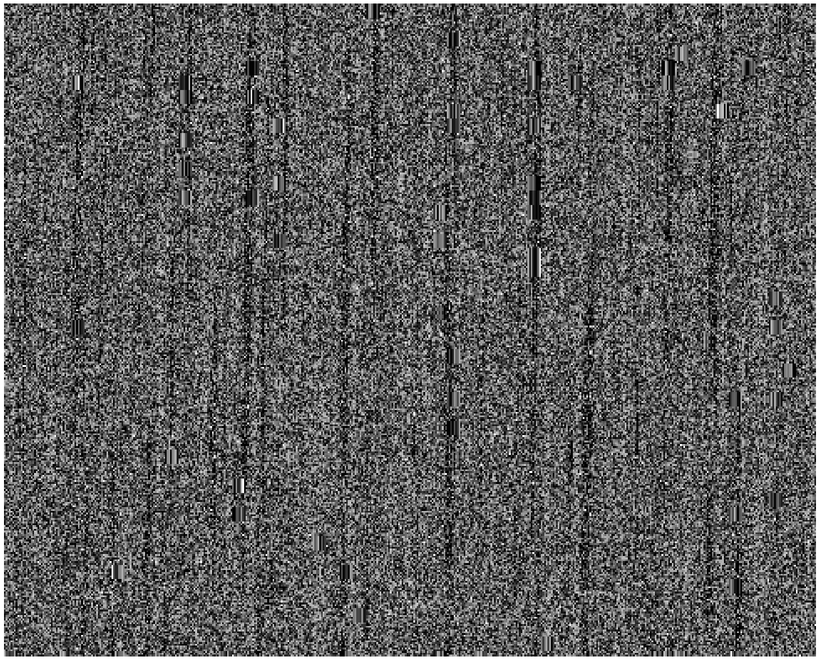
# Electrospinning- Key Issues

National University of Singapore

- How to produce nanofibers with controlled and uniform dimensions?
- How to place them in desired patterns and assemblies reproducibly?
- How to scale-up the process cost effectively?
- How to measure, predict, tailor and control physical, mechanical, chemical, biological, optical and electrical properties?

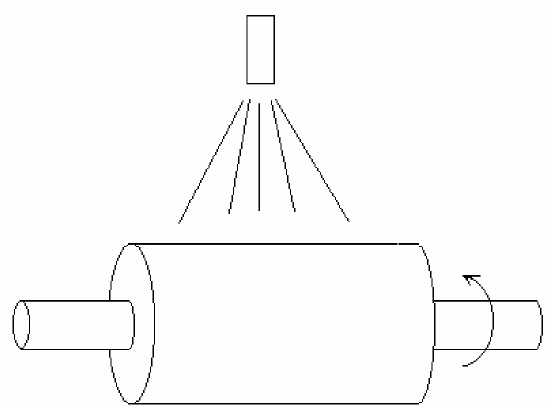
# Aligned Nanofibers ?



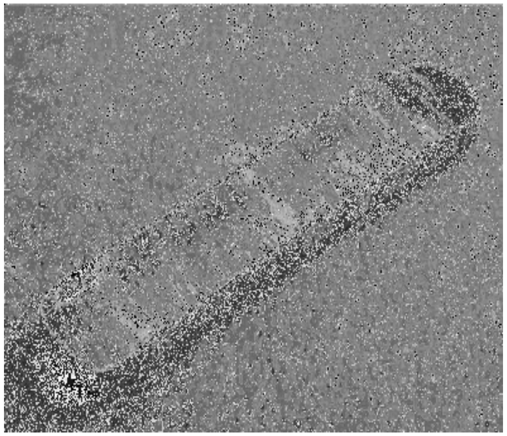
# Aligned Fibers

**Cylinder collector**



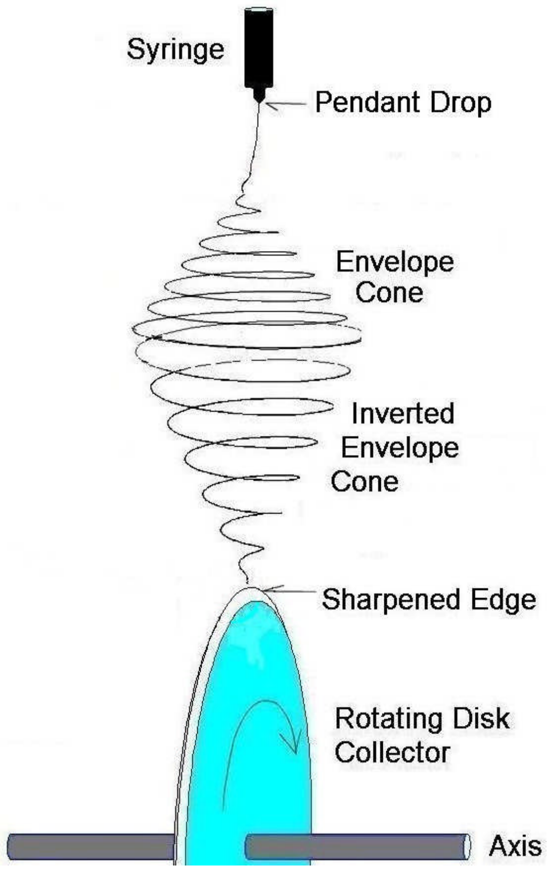
Boland et al., J Macromol Sci Pur Appl Chem, 2001

**Frame collector**



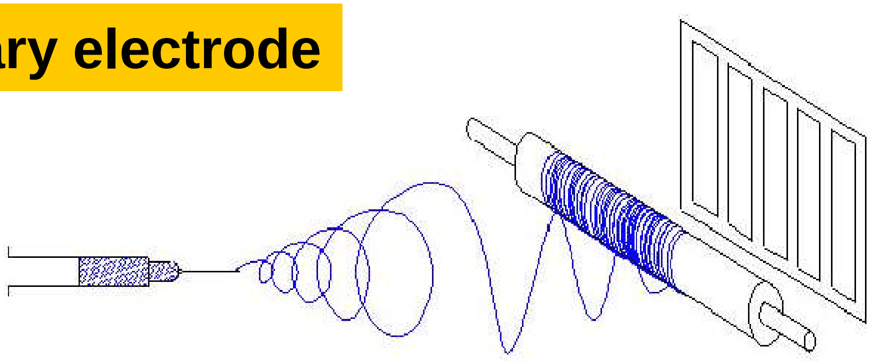
Dersh et al., J. Polym. Sci. B Polym. Phys., 2003

**Disk collector**




Theron et al., Nanotechnology, 2001

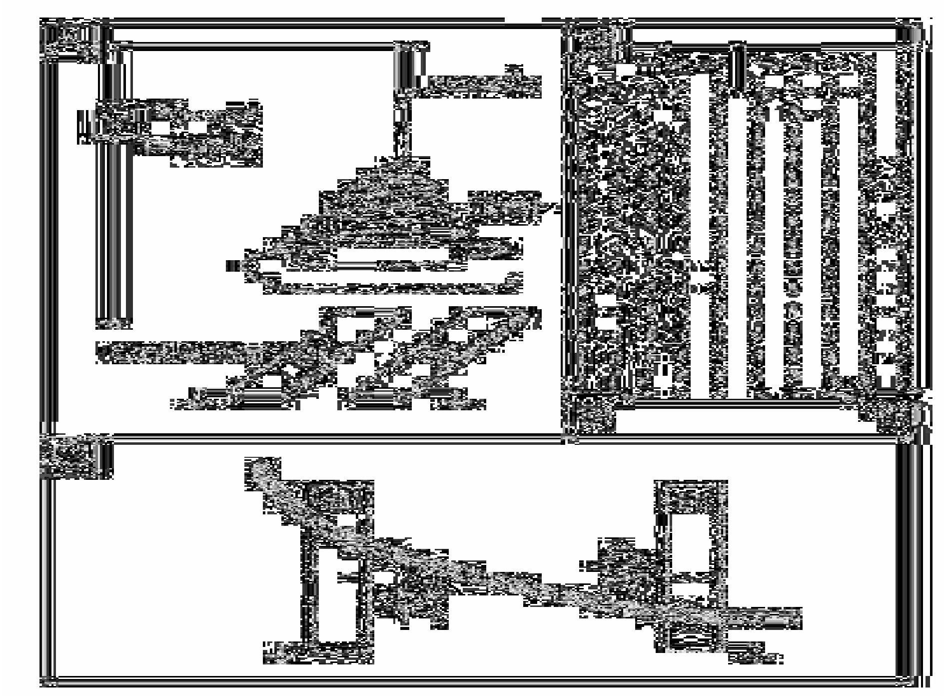
**Auxiliary electrode**



Bornat, US Patent, 1987

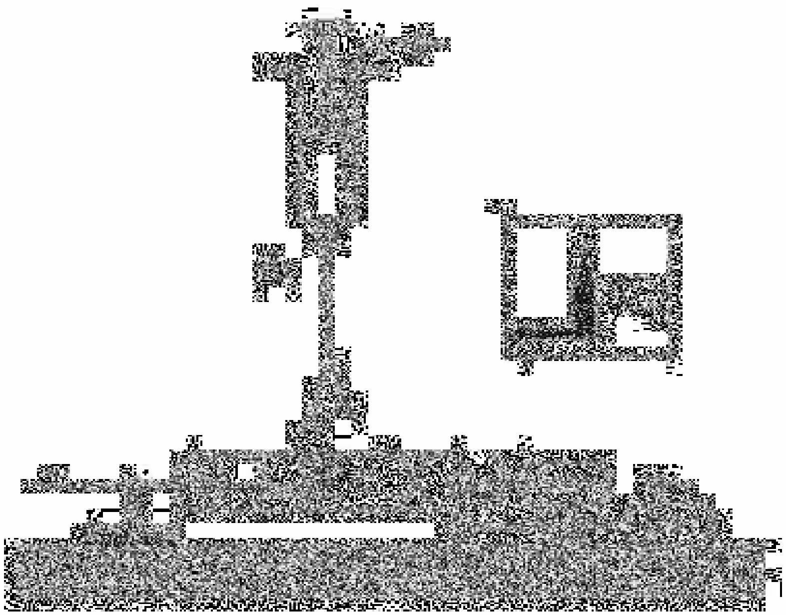
# Aligned Fibers

**Parallel electrode**



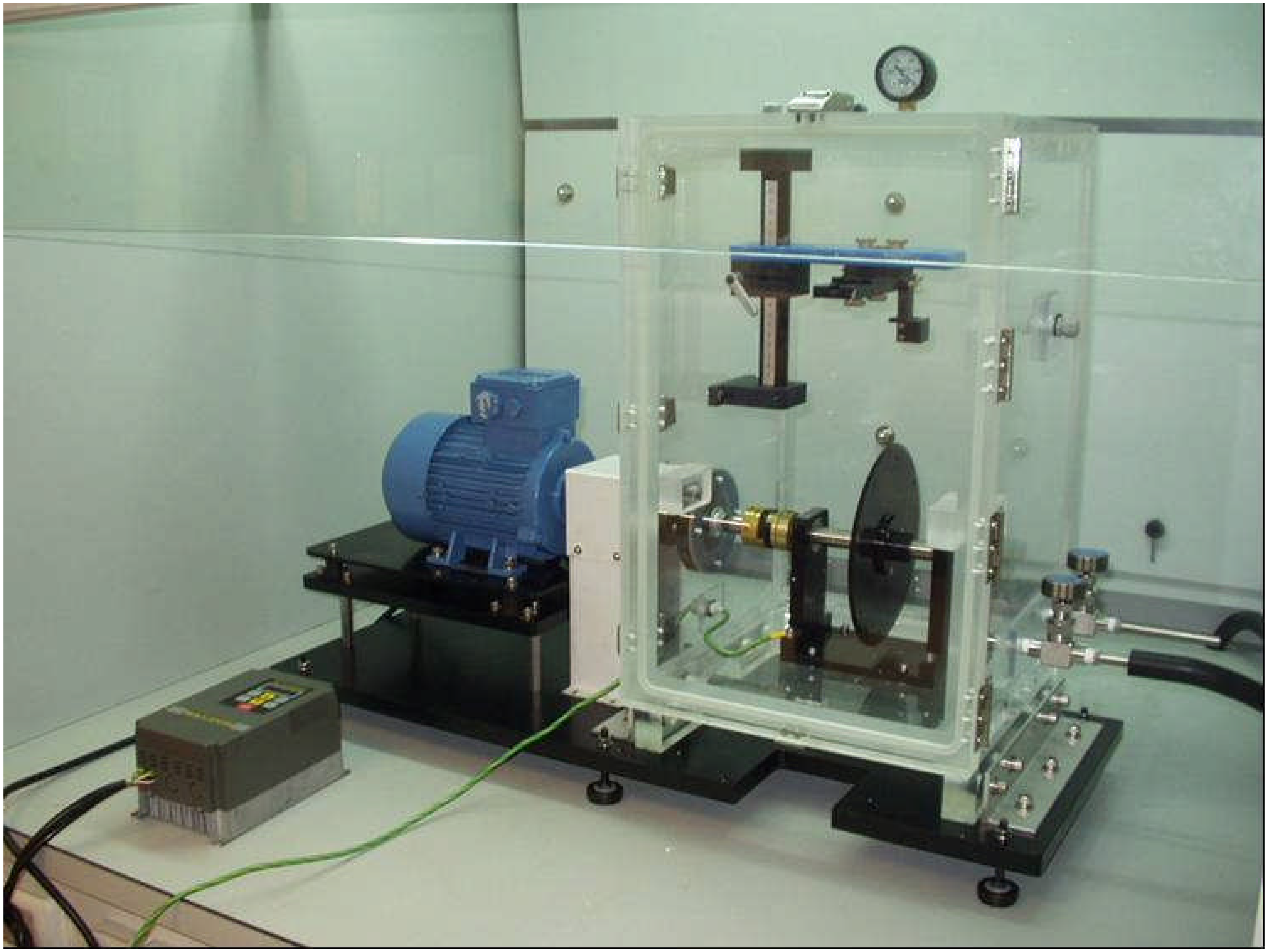
Dan Li et al., Nano Letters, 2003

**Sharp pin counter electrode**

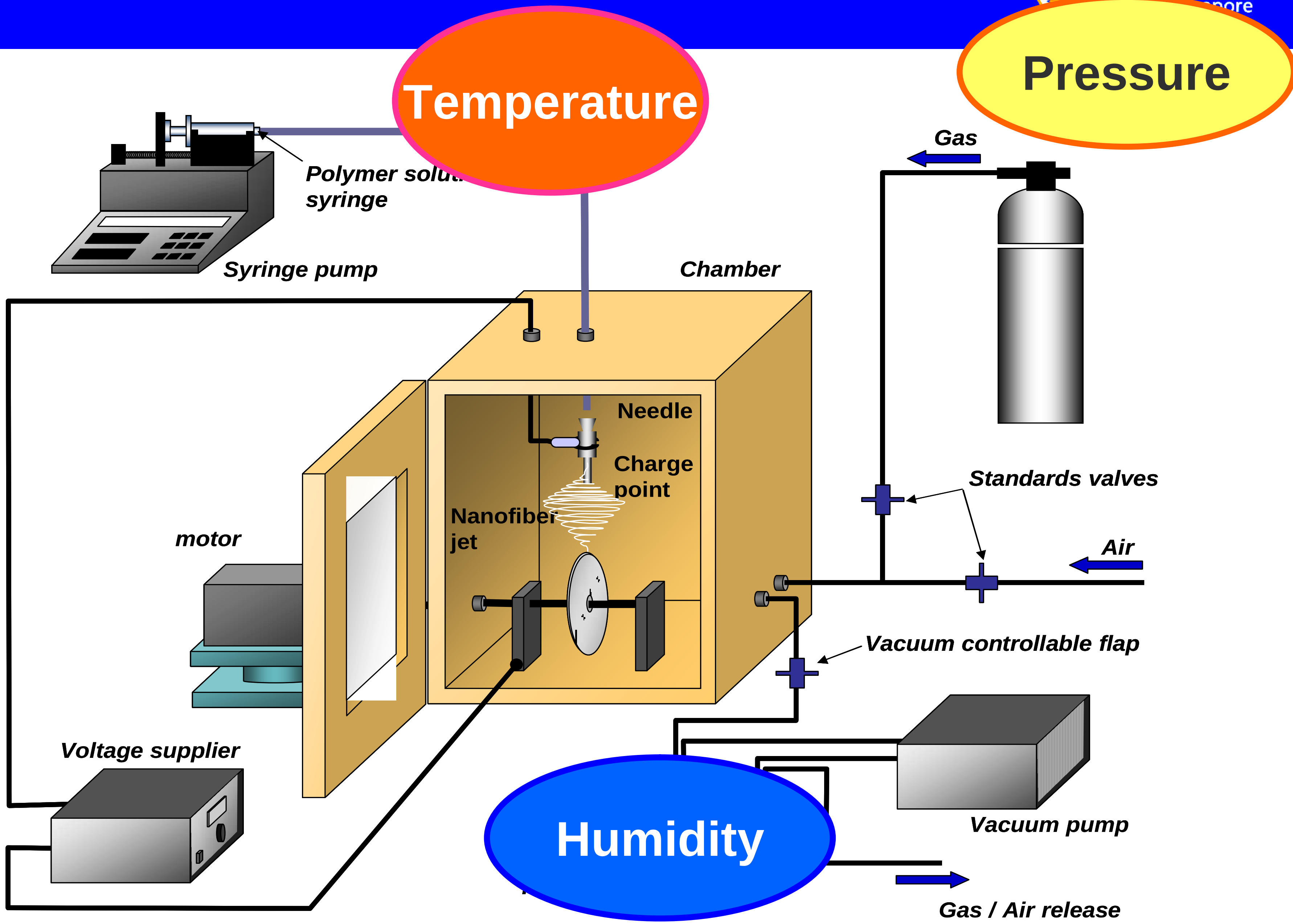


Natarajan et al., Applied Physics Letters, 2004

# Custom Built E-spinning Set-up

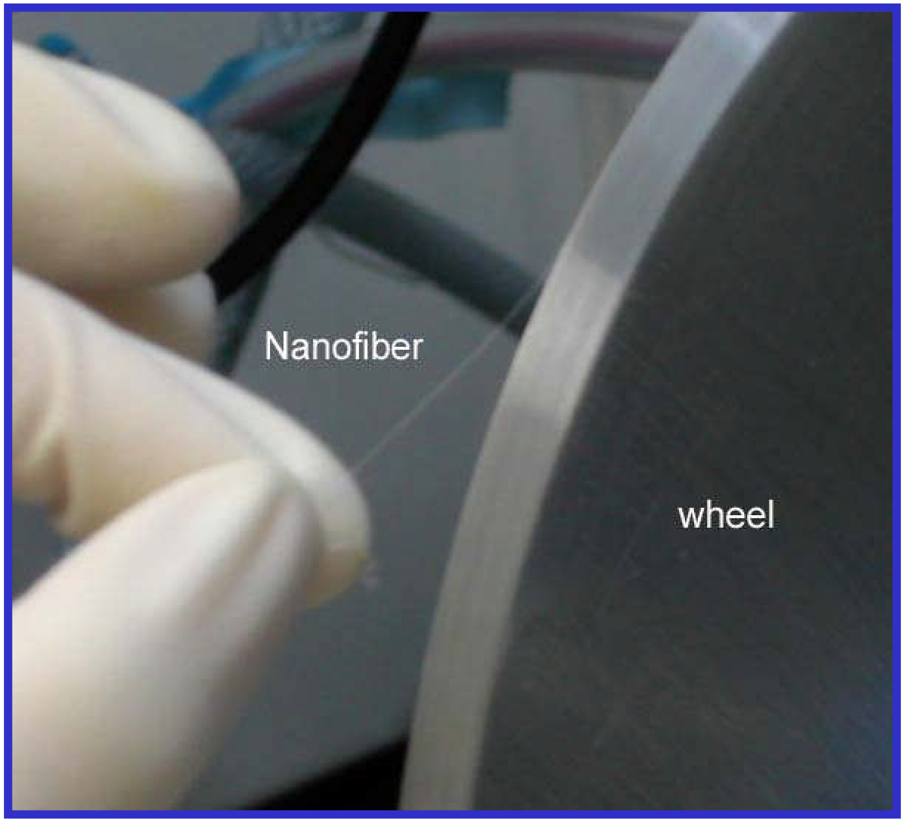


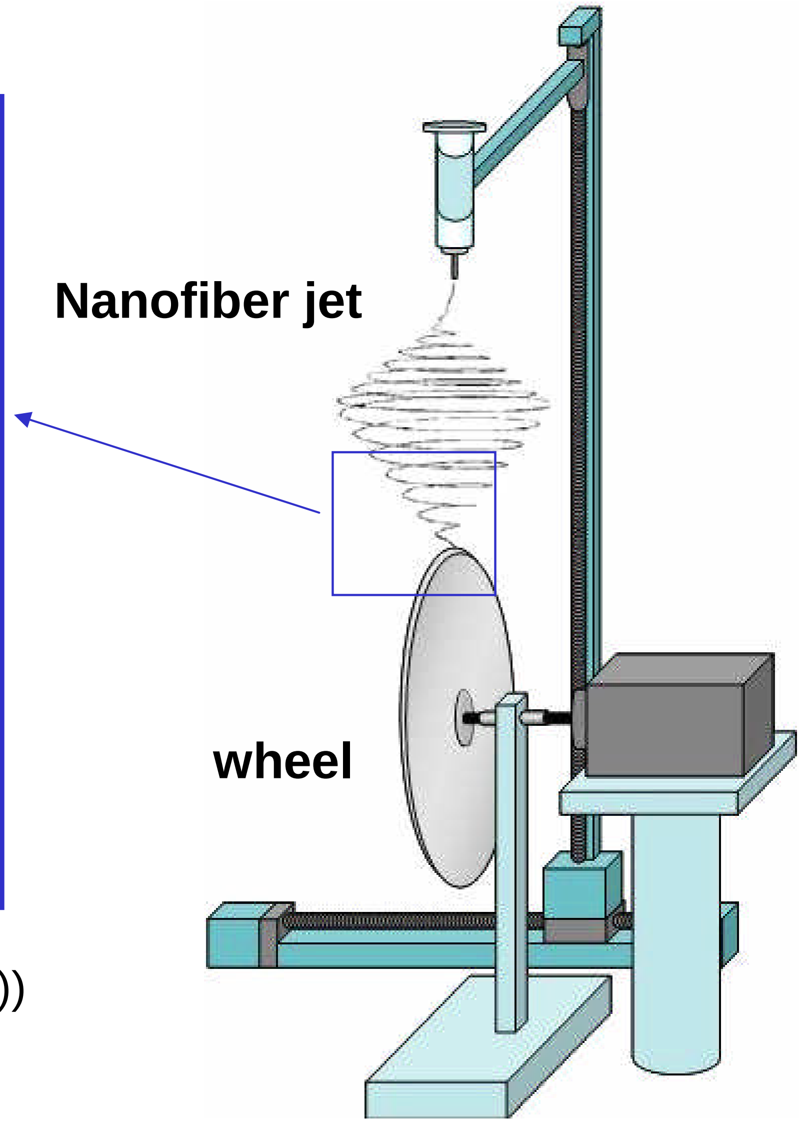
# Enhancement of E-Spinning Set-up




ICNT, Nov 7-11, 2004

# Nanofiber Bundle



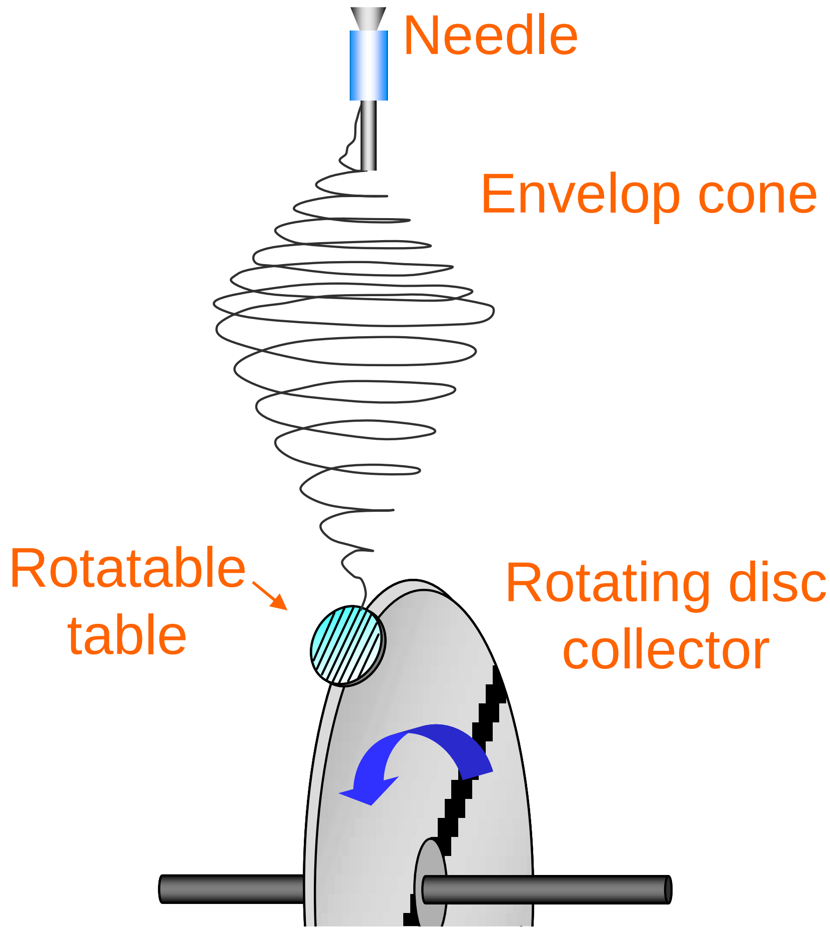




PCL 10w% (dichloromethane/ methanol (75/25))
- Voltage: 10kV
- Disk rotation speed: 11m/s

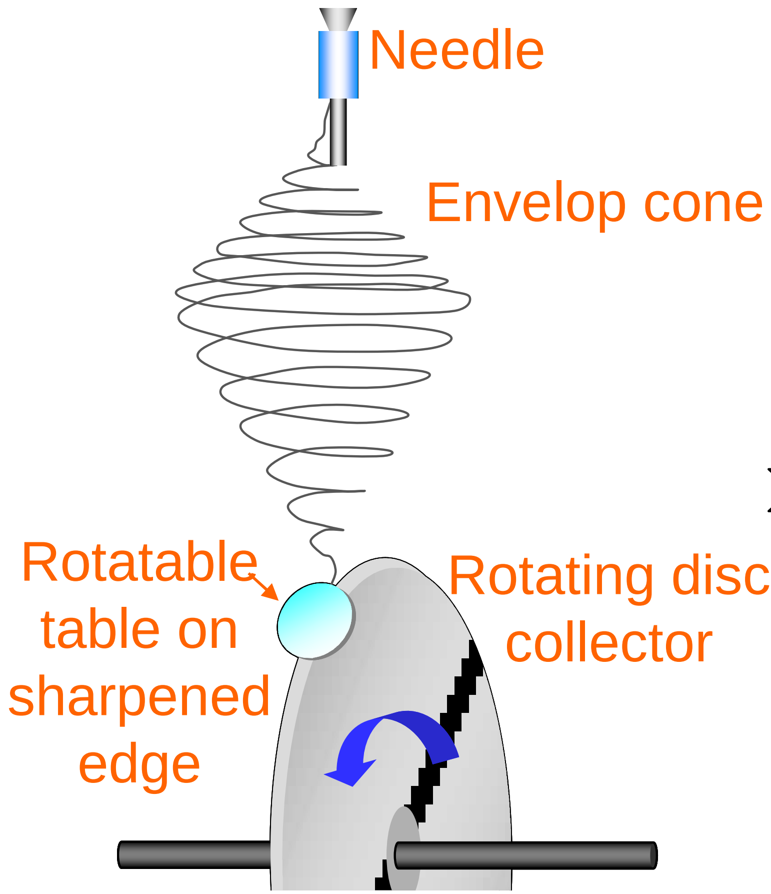
Seeram et al. Innovation, 2003

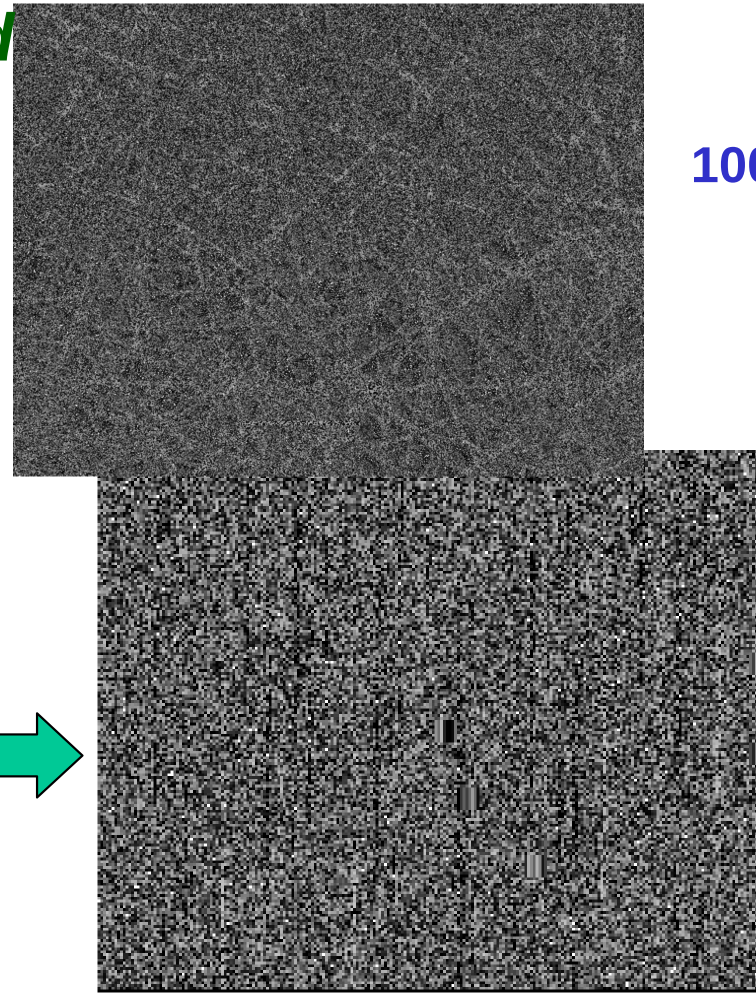
# Preliminary Results

- ***Collector Design***



# *Effect of Disc Rotation Speed*



**100rpm**

**1,000rpm**
**2D Aligned**

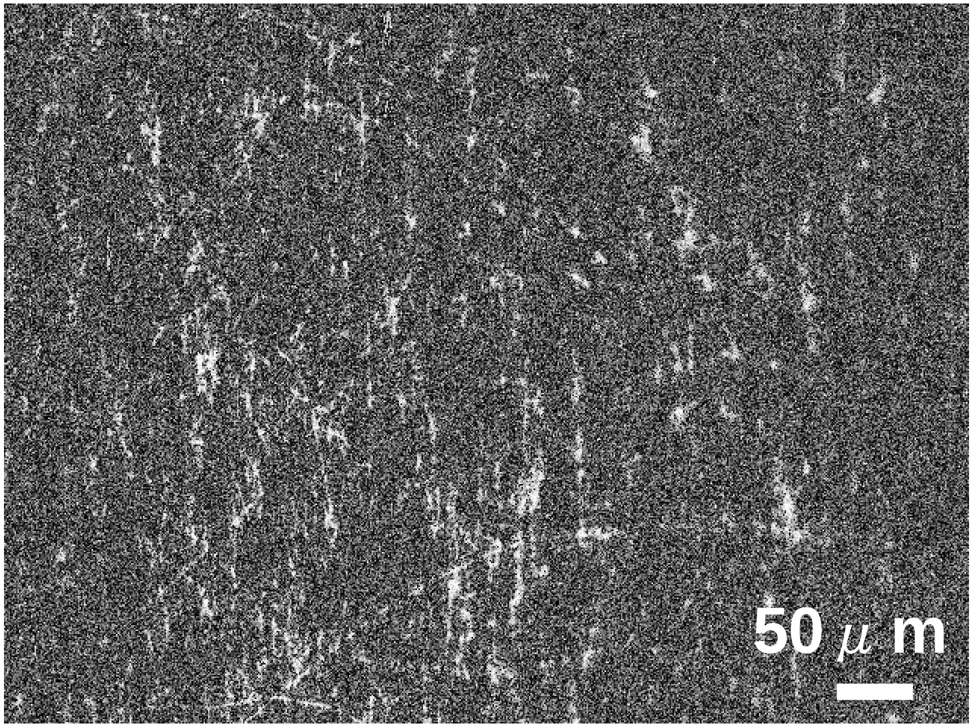
PCL10wt% (dichlomethane75%-methanol25%)
Voltage: 10kV

Seeram et al. Innovation, 2003

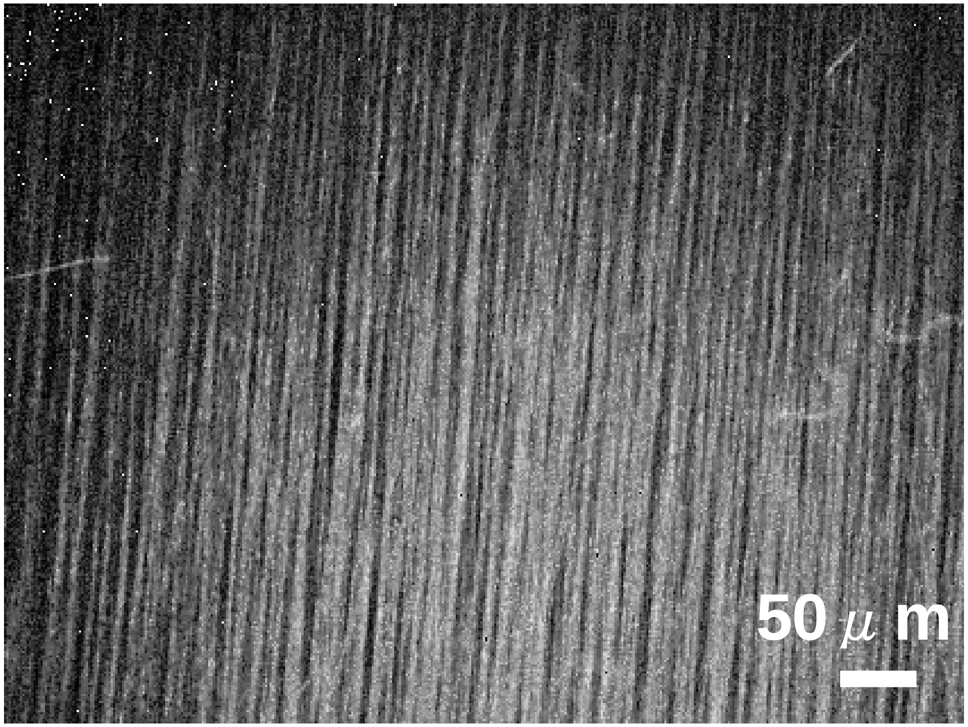
# Preliminary Results

NUS National University of Singapore

## *Effect of Material of Rotatable Table*



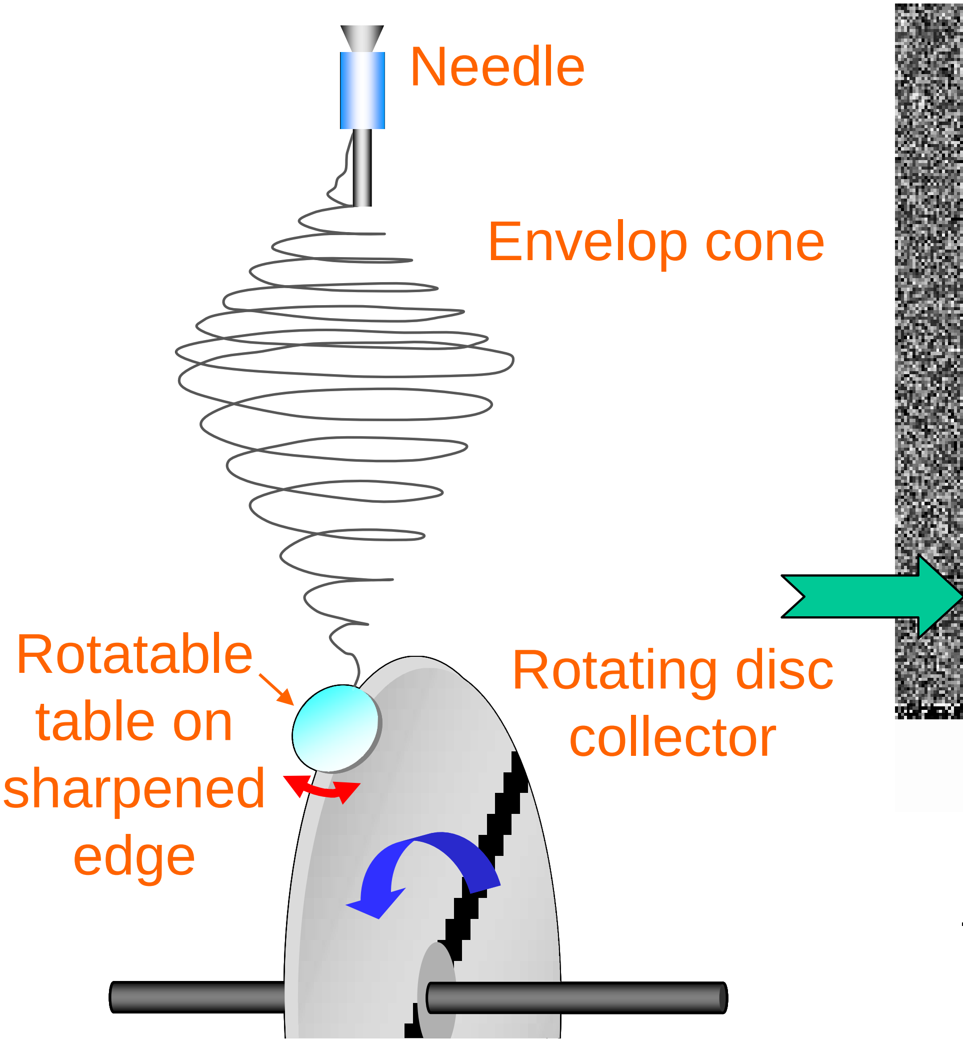

Conductive

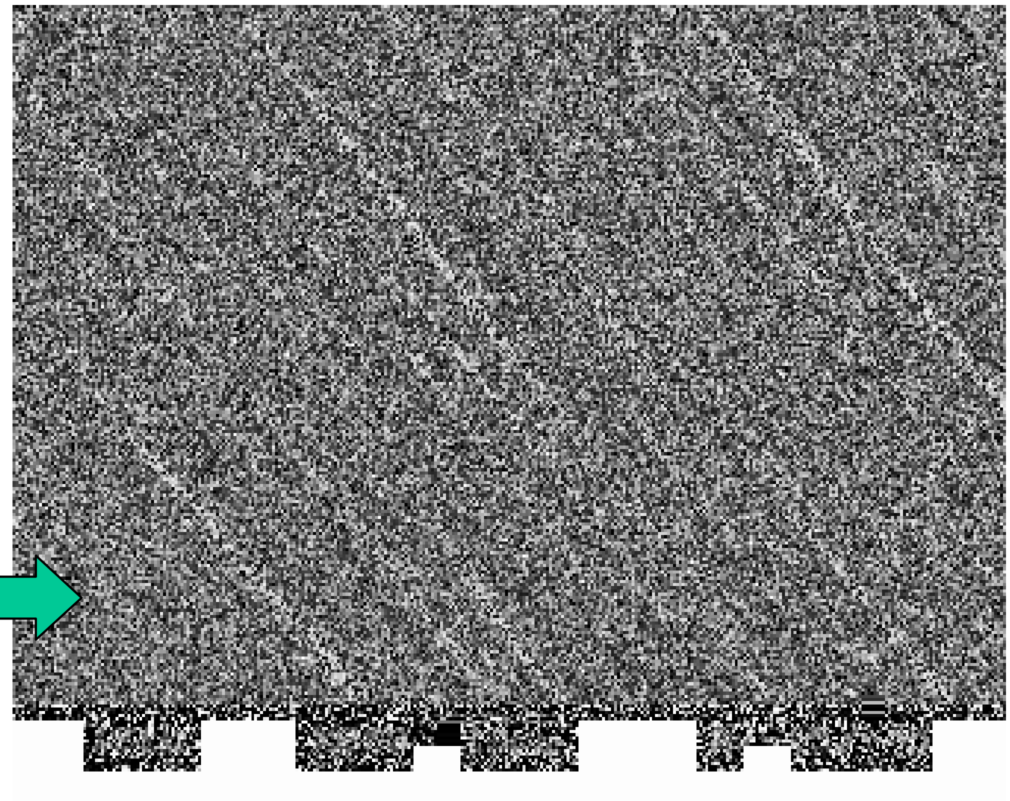



Non-conductive

# *Effect of Orientation of Rotatable Table*

## 3D Structure (0º/45º/-45º)

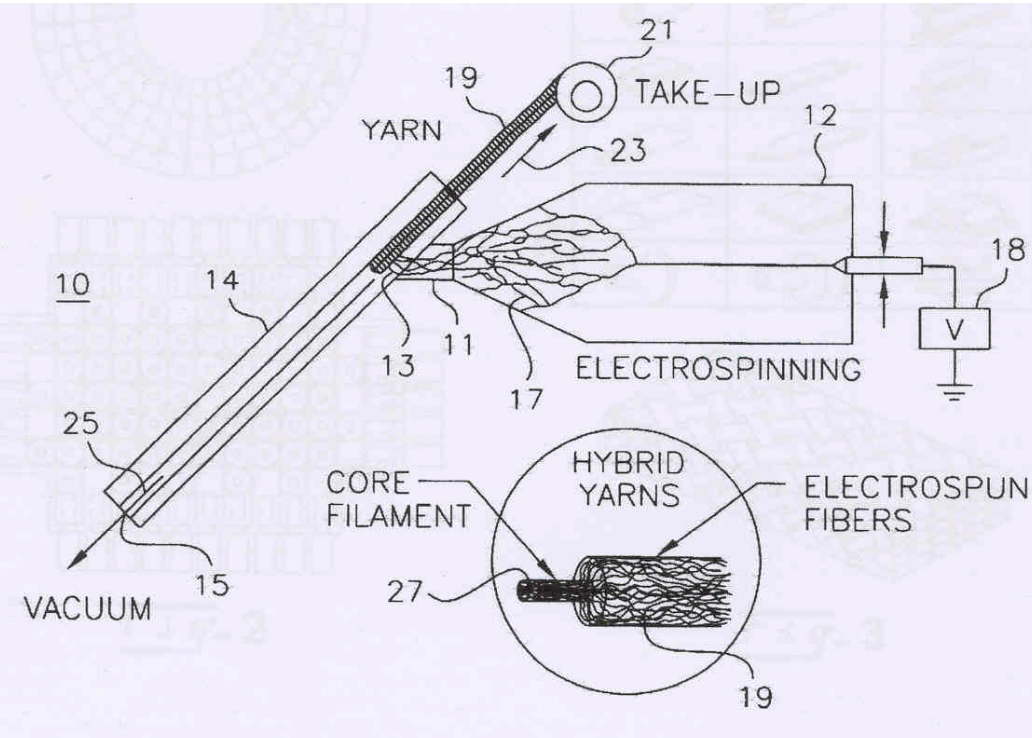


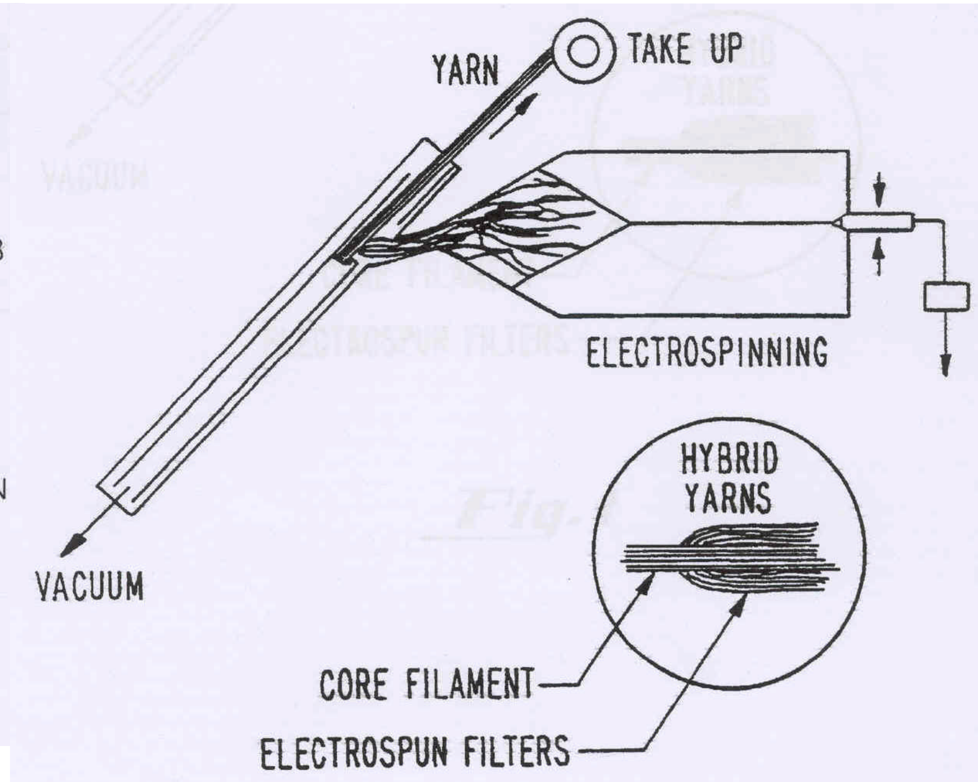
PCL10wt% (Chloroform75%-methanol25%)
- voltage: 10kV, - disk rotation speed: 11m/s

Seeram et al. Innovation, 2003

# Nanofiber Yarns



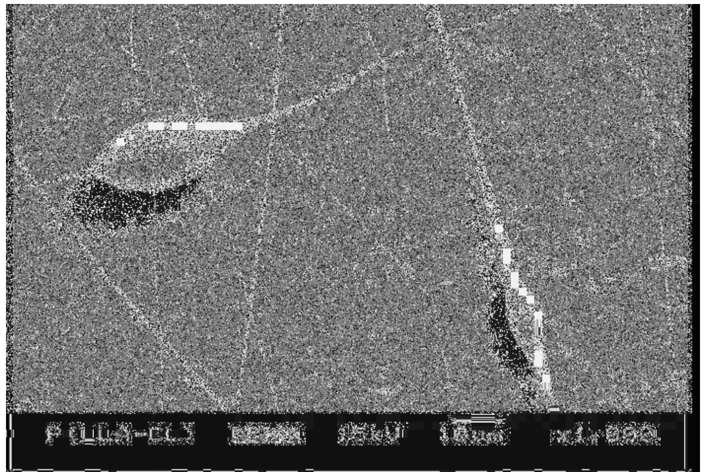

Scardino et al US Patent 6,308,509 Oct 30, 2001




Laurencin et al US Patent 6,689,166 Feb 10 2004

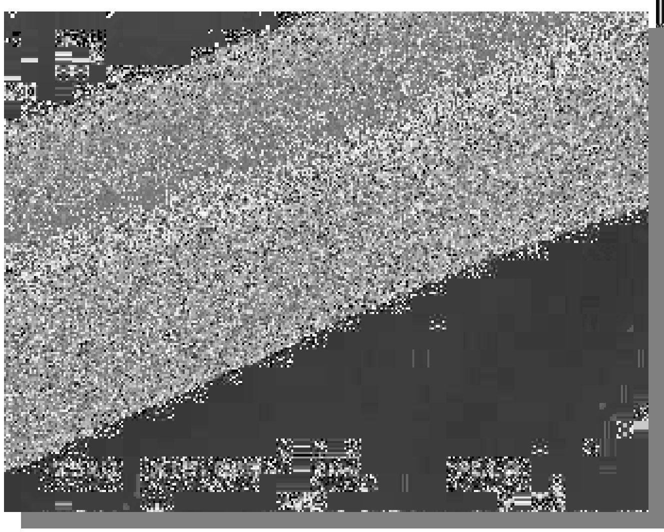
# Other Fiber Morphologies

## Beads formation



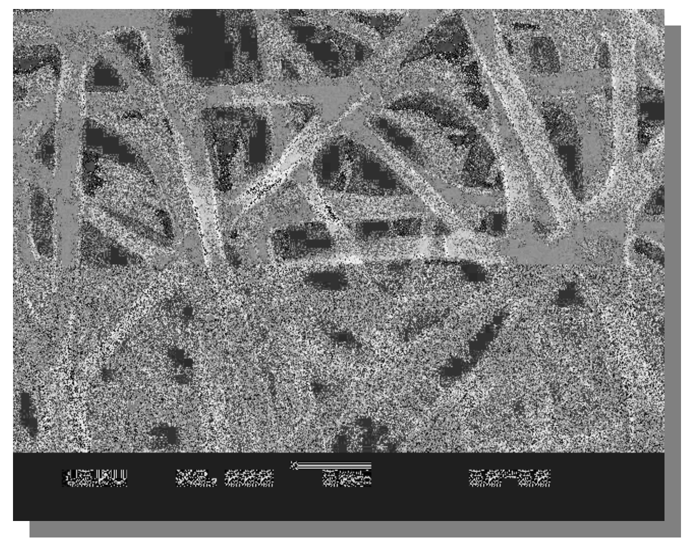
P(LLA-CL) 10w%
(dichlormethane/7.5phr palladium)
- Voltage: 10kV

## Porous Fibers



PLLA 7.5w%
(dichlormethane/dimethyl formamide)
- Voltage: 20kV
- Disk rotation speed: 11m/s

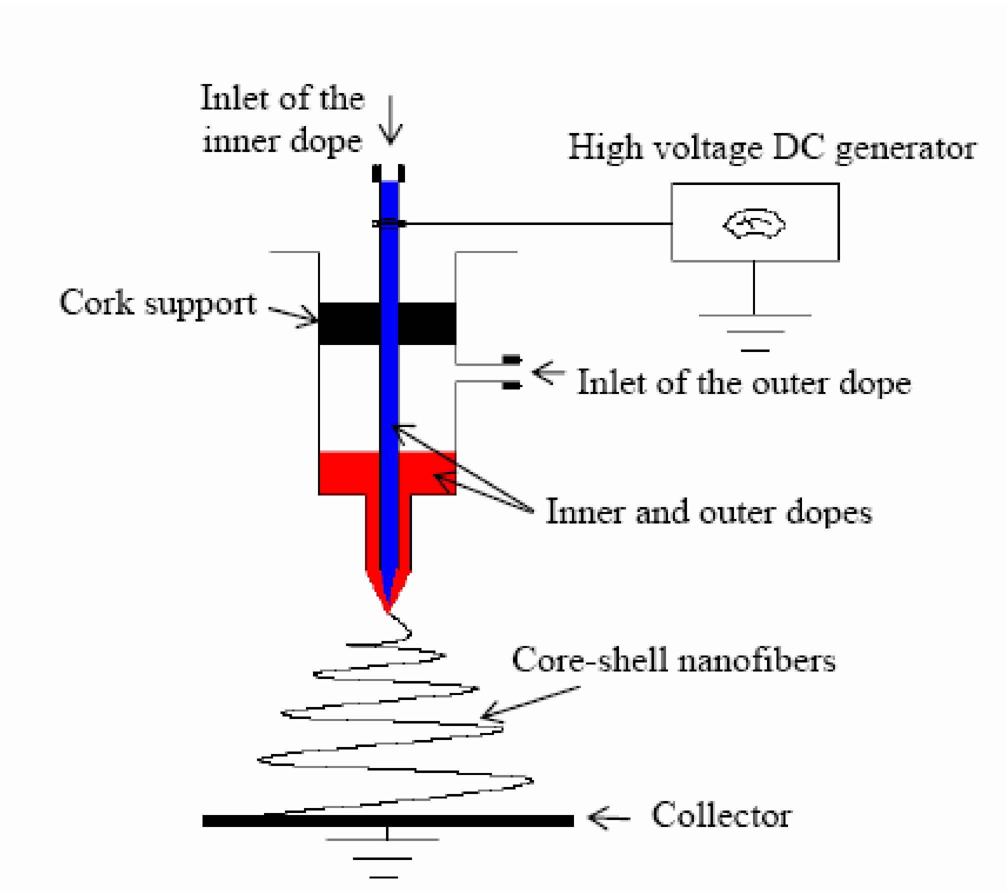
## Interconnected Structure



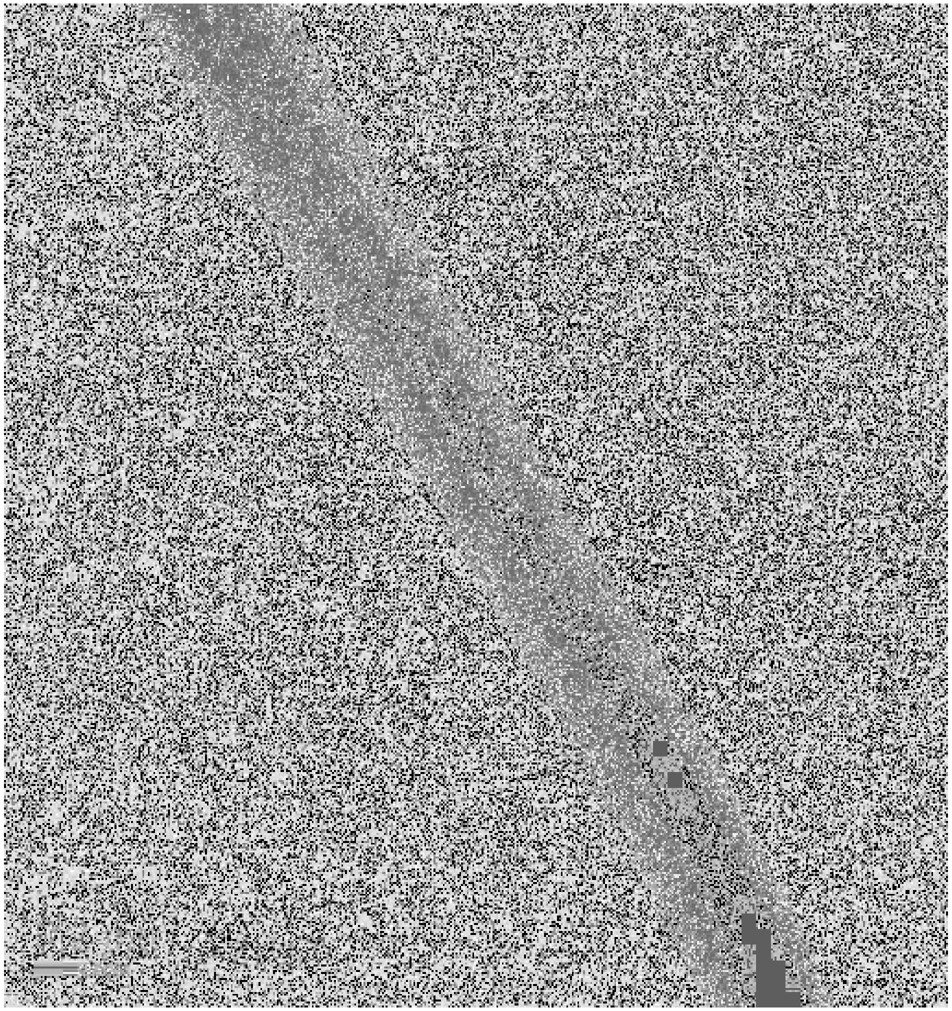
P(LLA-CL) 7.5w% (Acetone)
- Voltage: 15kV

# Shell-Core (& Hollow) Nanofibers

## Coaxial Electrospinning

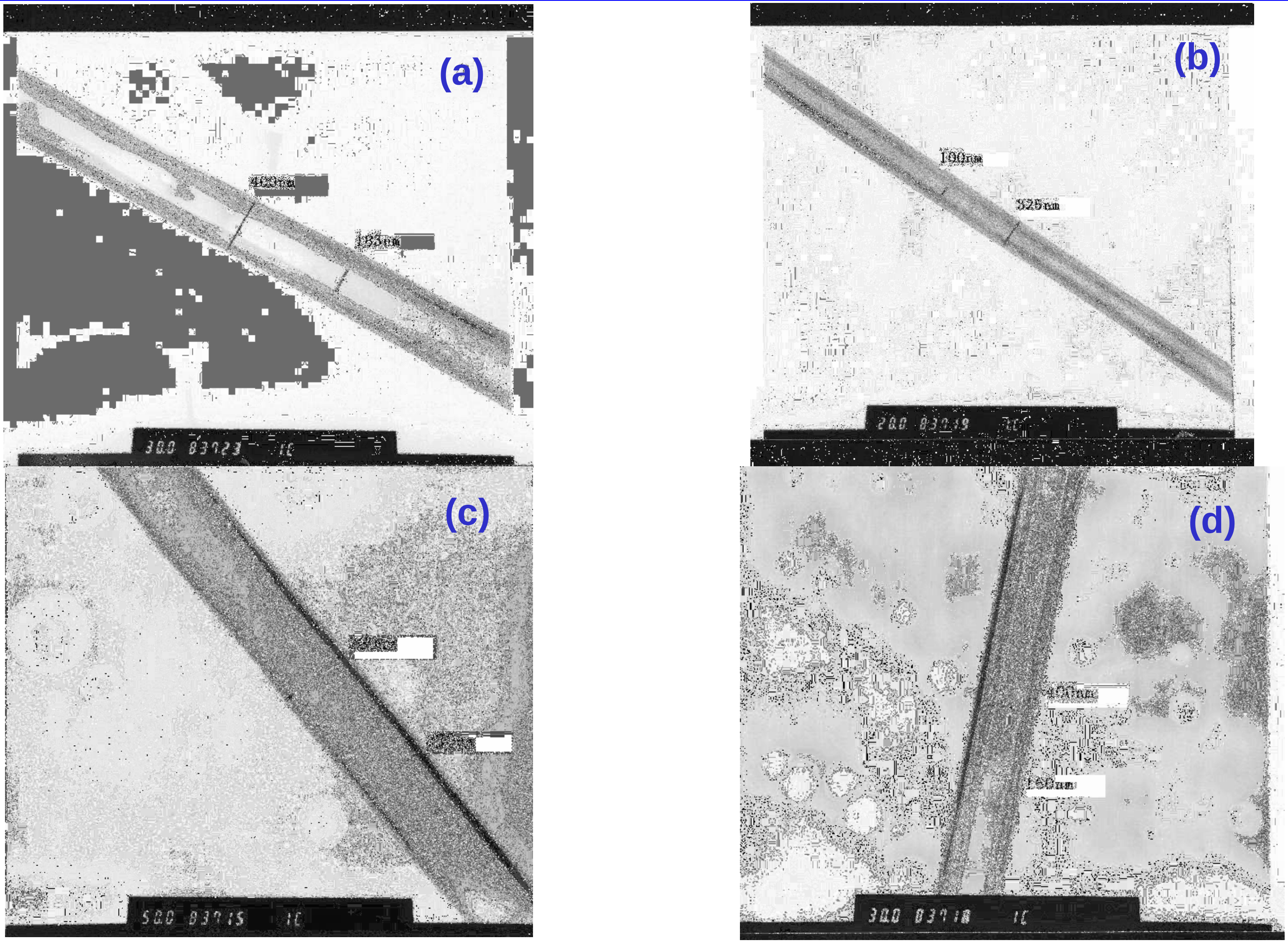



## PCL / Gelatin (shell / core)



ICNT, Nov 7-11, 2004

# PCL (shell) / Drug (core)

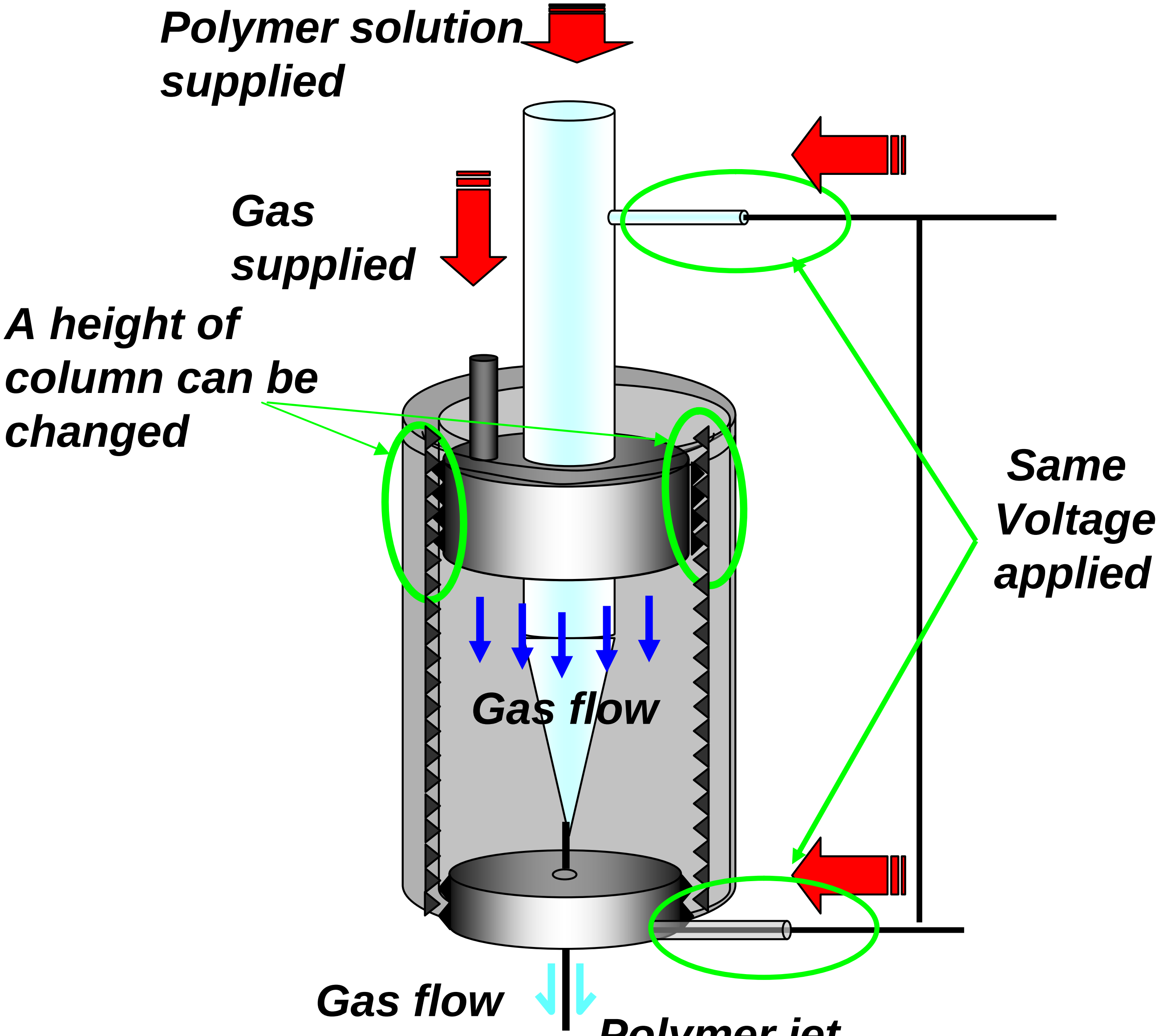



**TEM images of the shell-core compound nanofiber segments with the resveratrol concentrations of (a) 4%, (b) 6%, (c) 8%, and (d) 10% in the core and 7% PCL in the shell**

# Further Enhancement of E-Spinning Set-up

Schematic diagram of Spinneret with Jacket
(courtesy of Ryuji)

- The rate of gas flow along the jet , controls the degree of elongation jet experiences. The flowing gas *pulls* the polymer jet , by applying shear stresses on the latter's surface. Controlling the degree of jet elongation may allow for control over the uniformity and fineness of the fiber formed.



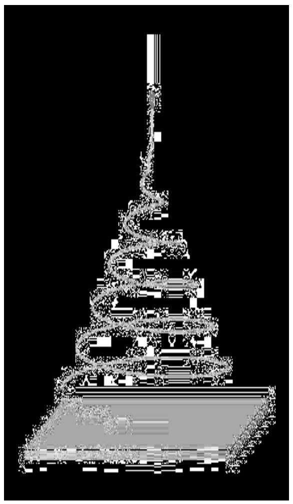
# Electrospinning- Key Issues

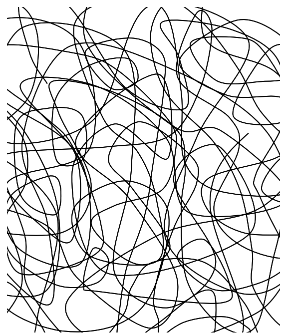
National University of Singapore

- How to produce nanofibers with controlled and uniform dimensions?
- How to place them in desired patterns and assemblies reproducibly?
- How to scale-up the process cost effectively?
- How to measure, predict, tailor and control physical, mechanical, chemical, biological, optical and electrical properties?

# Production Rate of Current Methods

Lab Scale Set-up





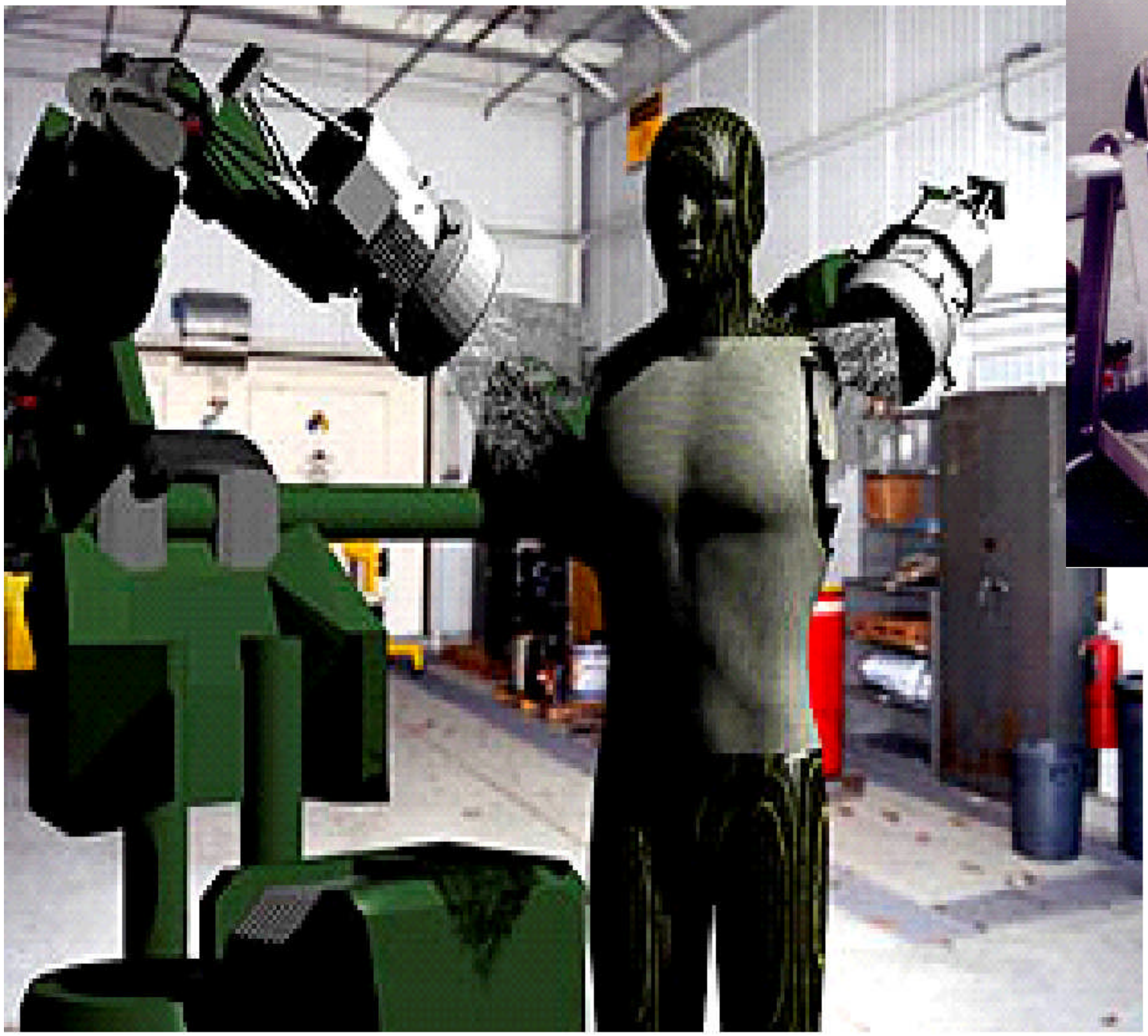
Random mats

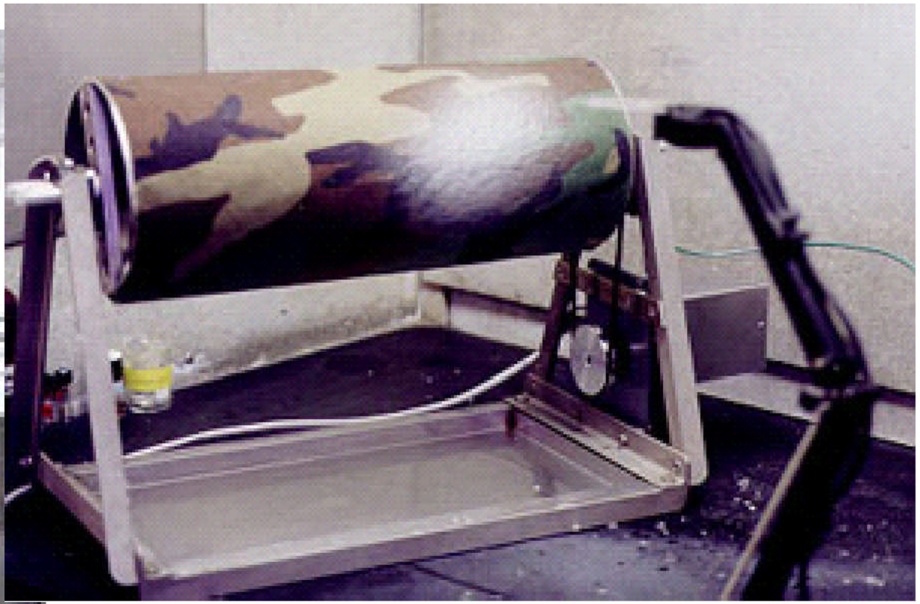
**Fiber Diameter = 300nm**

**Production Rate = 0.02g/jet/min**

“eSpin produces about 10,000 yards of nanofiber per day – far below the needs of major filtration companies. “We have one customer that would like to have 50 million square yards a year. That’s (only) one customer, and there are hundreds of filter companies.”

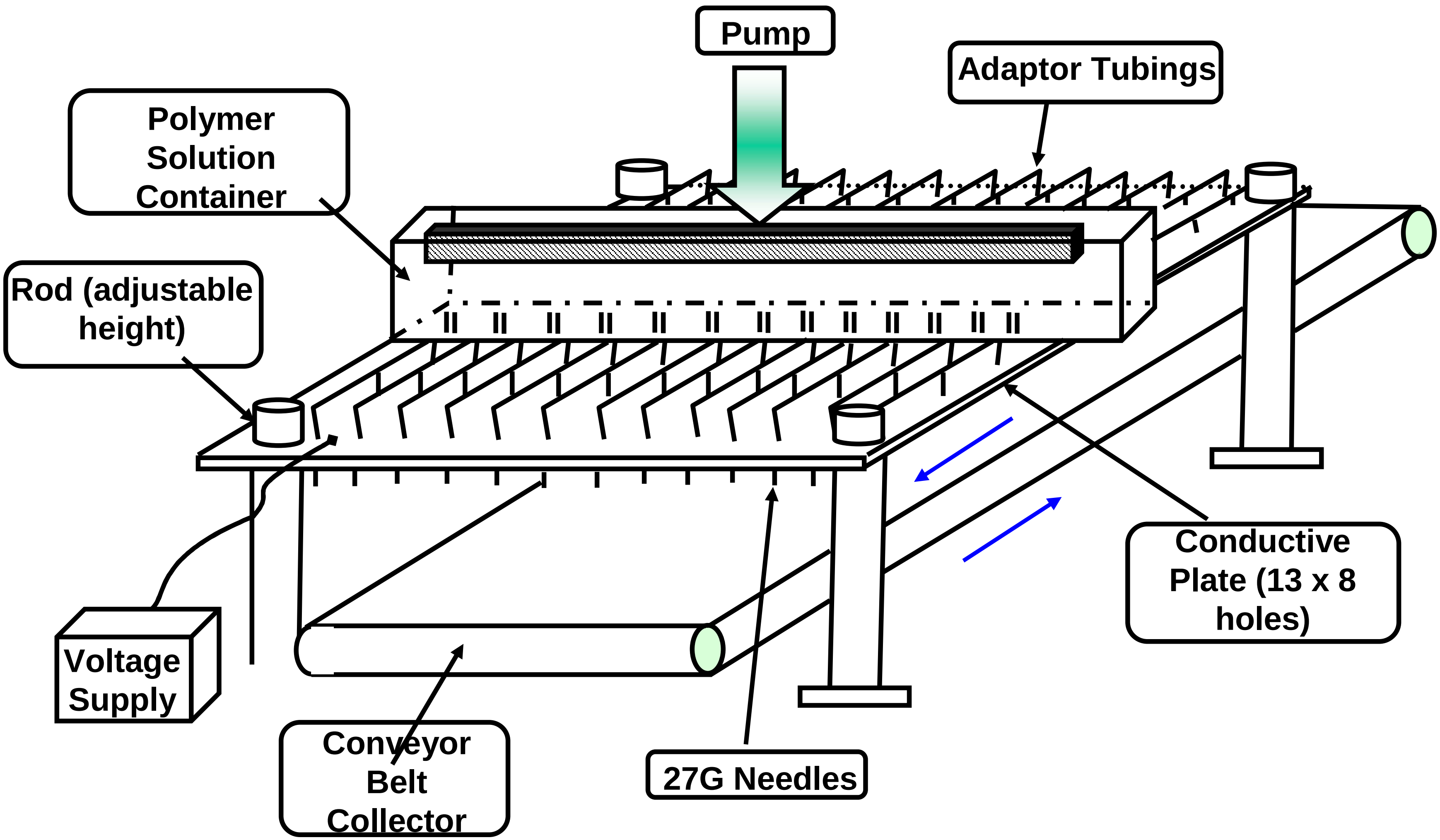
# Large Scale Electrospinning



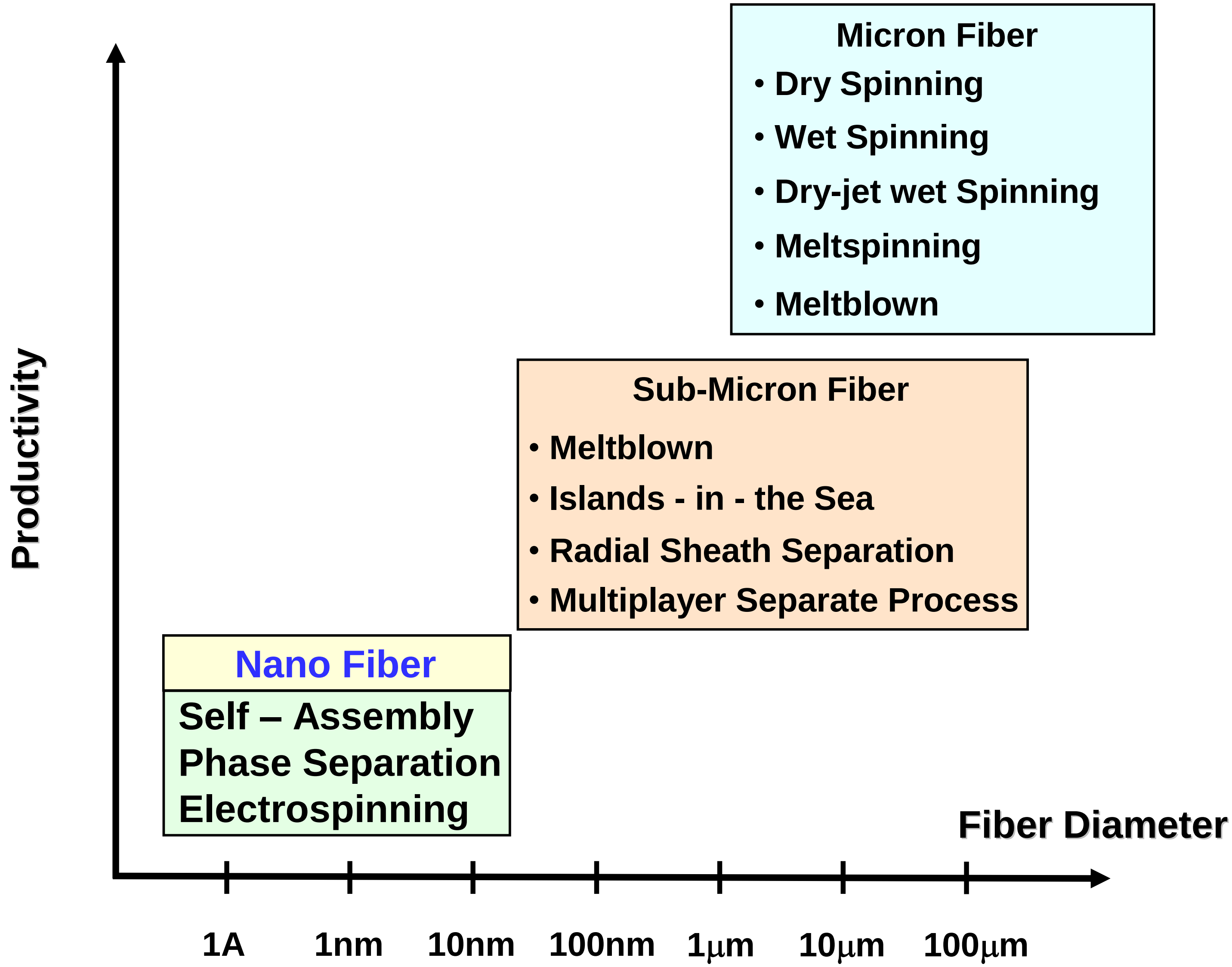


(reference: http://www.inteletex.com/FeatureDetail.asp?PubID=&NewsId=1462)

# Multiple Jet Electrospinning



# Productivity of Processes




*Adopted from Rutledge @ MIT*

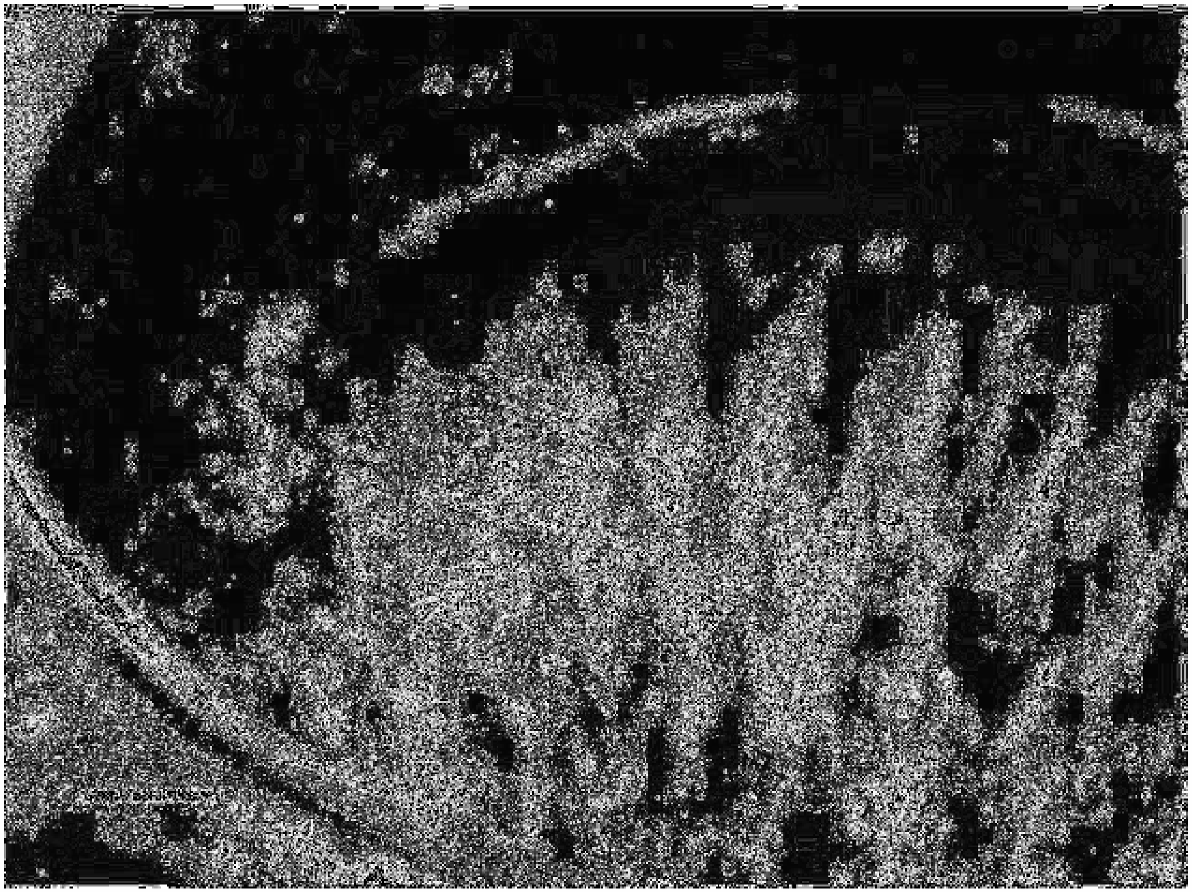
# Multiple Jet Spinning

- How do the jets influence one another?
- How can the number of applied jets be maximized with optimum spacing?
- How to better control jet formation and acceleration?
- How to achieve both high production rate and tailored lay-up of fibers

# Upward Needleless Electrospinning

NUS
National University of Singapore

## Thousands of upward jets



Yarin et al., Polymer, 2004

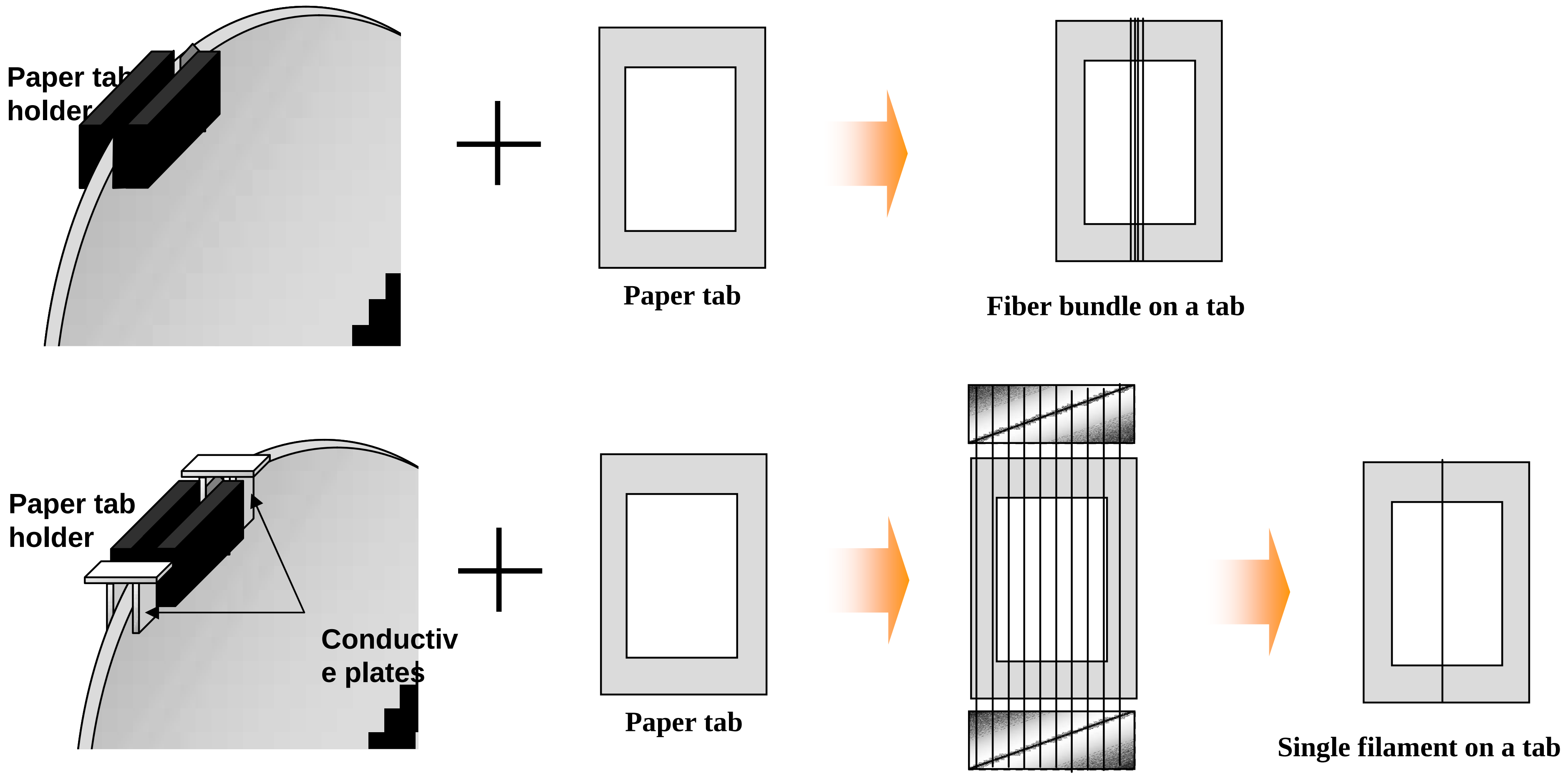
Multiple jets attracted to a piece of a metal saw used as a counter-electrode

# Electrospinning- Key Issues

NUS National University of Singapore

- How to produce nanofibers with controlled and uniform dimensions?
- How to place them in desired patterns and assemblies reproducibly?
- How to scale-up the process cost effectively?
- **How to measure, predict, tailor and control physical, mechanical, chemical, biological, optical and electrical properties?**

## Tensile Sample Preparation



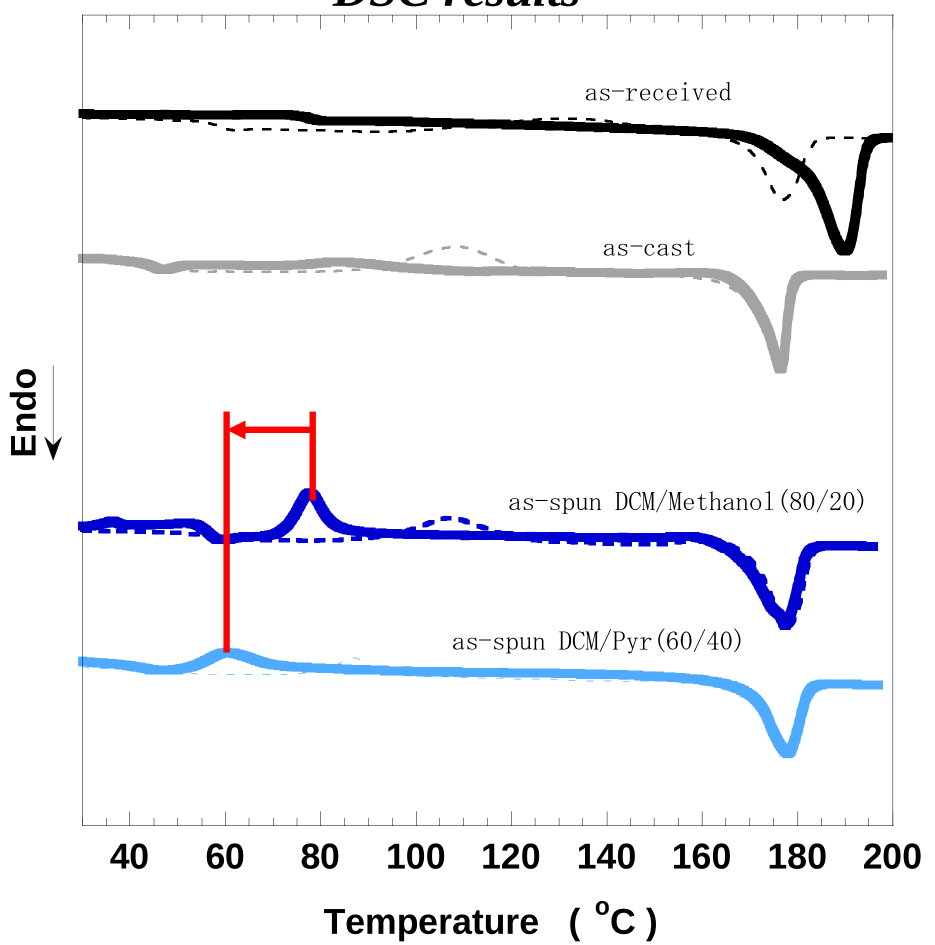
# Structure - Property Studies

NUS National University of Singapore

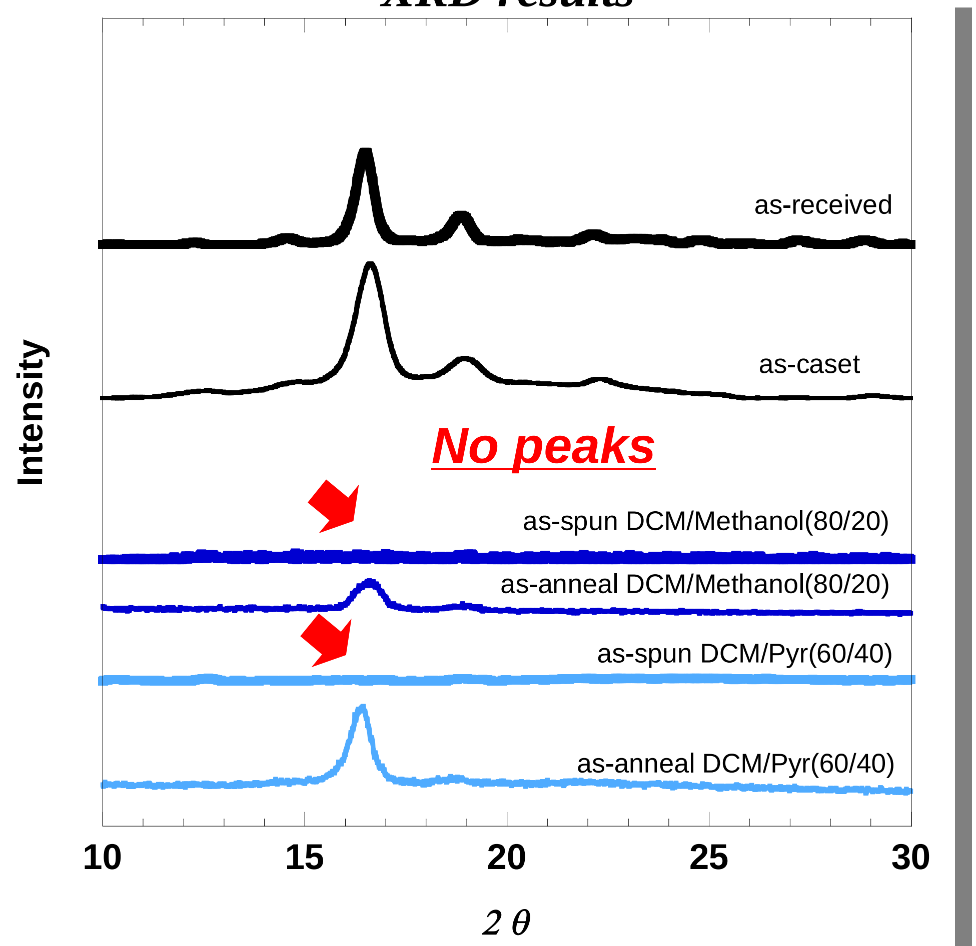
## *Solution conductivity effects*

**Higher conductivity ➡ Molecular orientaion**

*- DSC results -*




*- XRD results -*



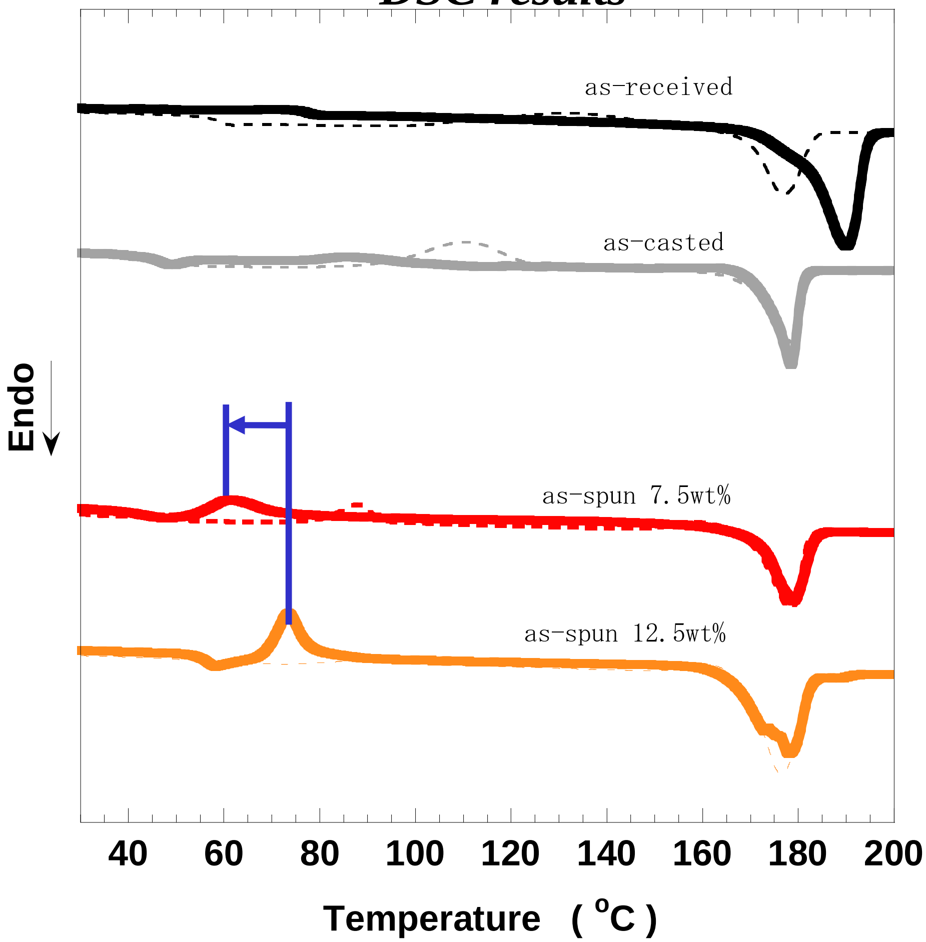
# Structure - Property Studies

National University of Singapore

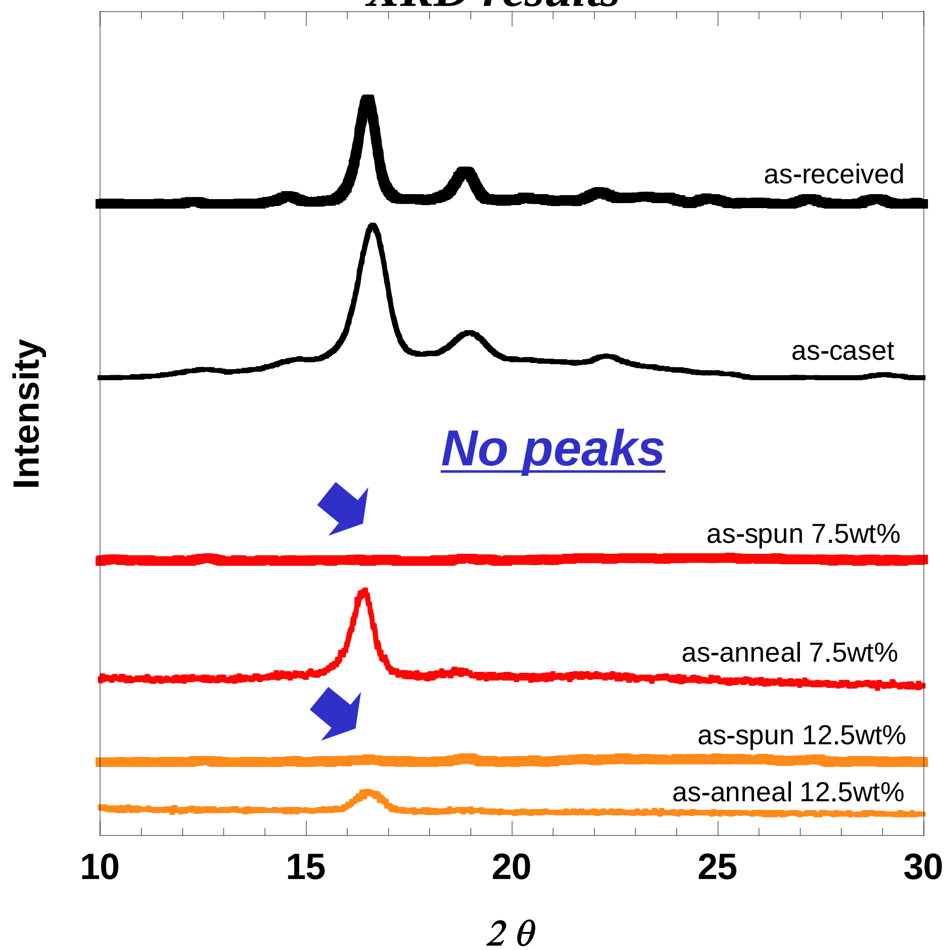
## *Polymer concentration effects*

**Lower concentration ➡ Molecular orientation**

*- DSC results -*




*- XRD results -*

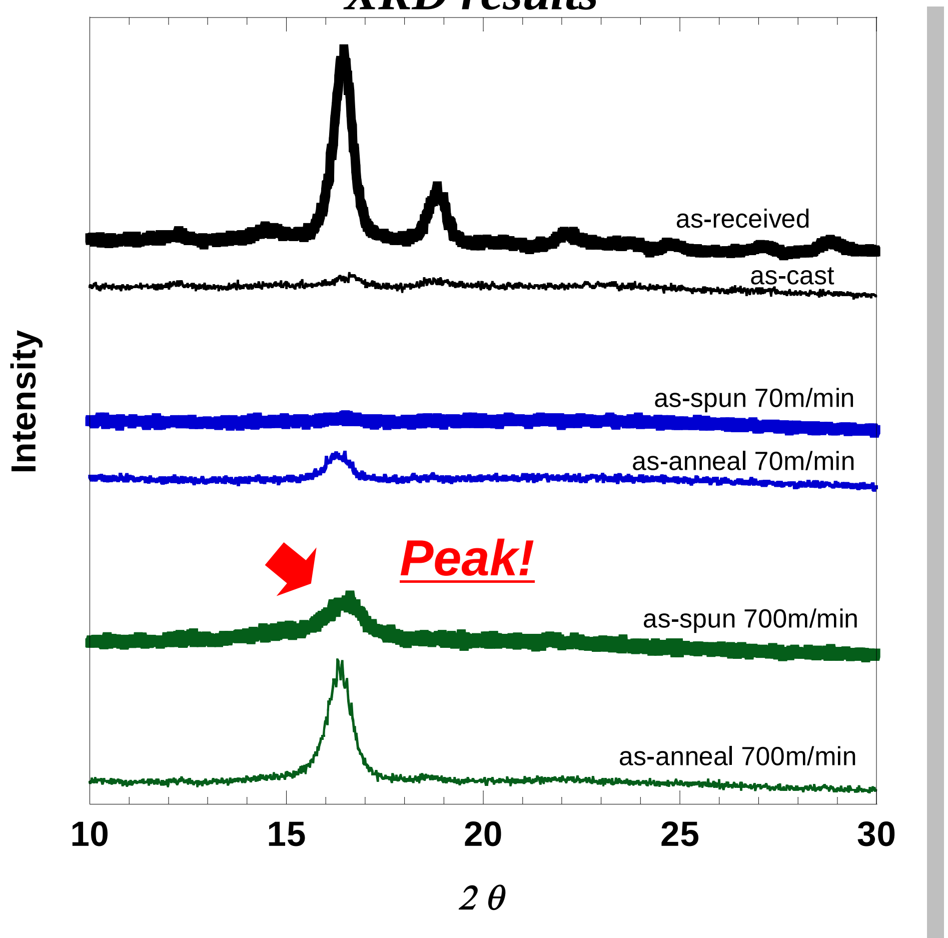


# Structure - Property Studies

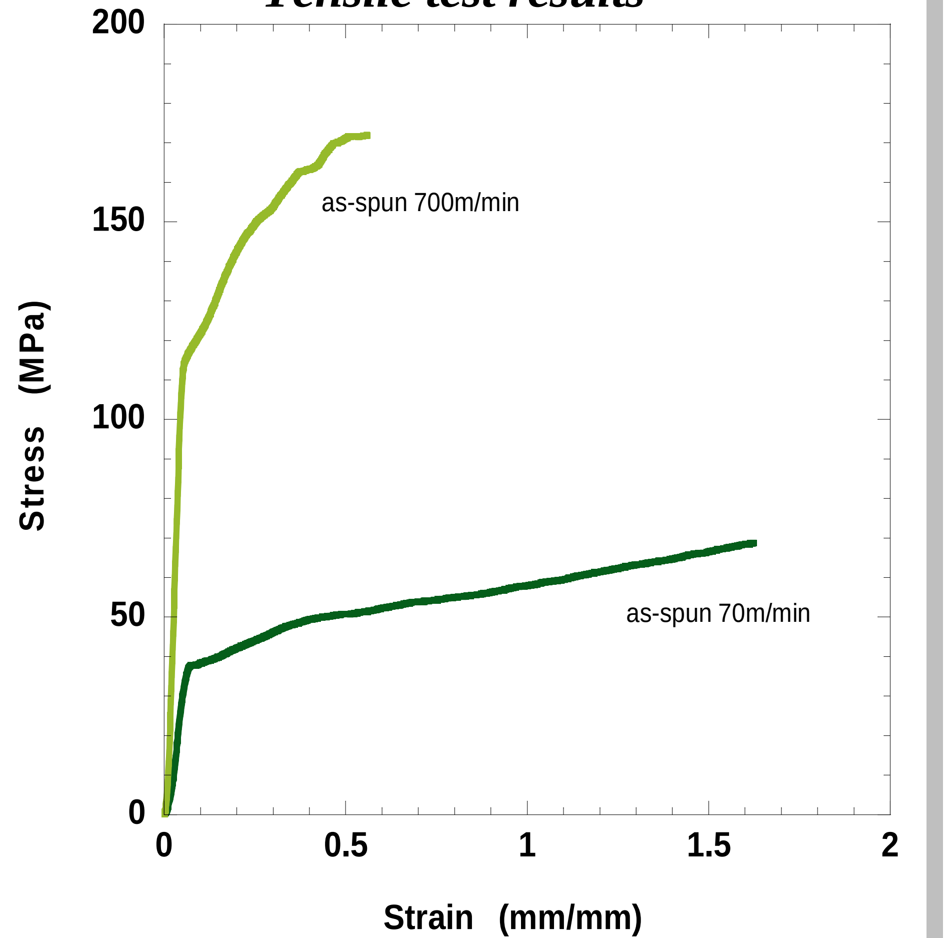
## *Take-up velocity effects*

**Take-up velocity ➡ Dominant parameter!!**

*- XRD results -*




*- Tensile test results -*



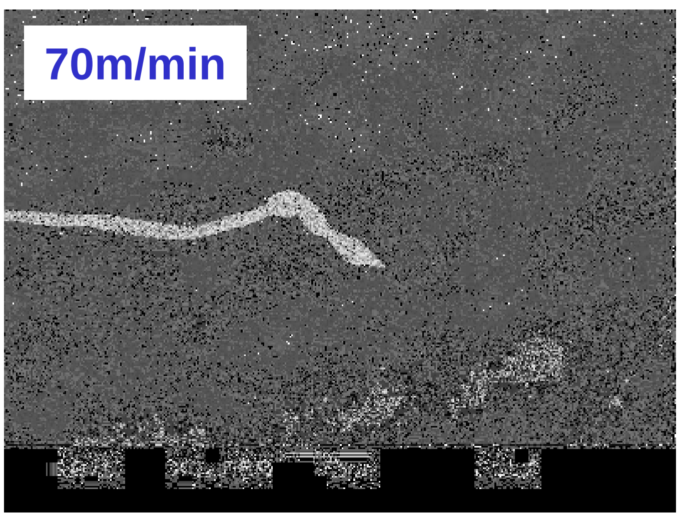
# Tensile Property

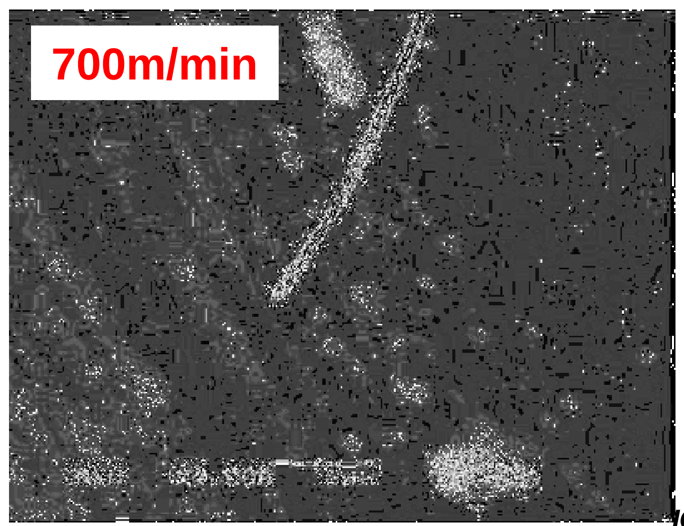
NUS
National University of Singapore

## PLLA single nanofiber

| Conditions | Tensile strength [MPa] | Tensile modulus [GPa} | Elongation at break [%] | Fiber diameter | notes |
|---|---|---|---|---|---|
| Film | 28-50 | 1.2-3.0 | 6.0-2.0 | | |
| Melt-spun fiber | up to 870 | up to 9.2 | 25 | several tens μm | spun & drawn |
| Solution-spun fiber | up to 2,300 | up to 16 | 12-26 | several tens μm | spun& drawn |
| Electrospun nanofiber | 89 ± 40.3 | 1.02 ± 1.6 | 1.54 ± 0.116 | 890 ± 190 nm | spun at 70m/min |
| | 183 ± 25 | 2.9 ± 0.435 | 0.454 ± 0.11 | 610 ± 50 nm | spun at 700m/min |

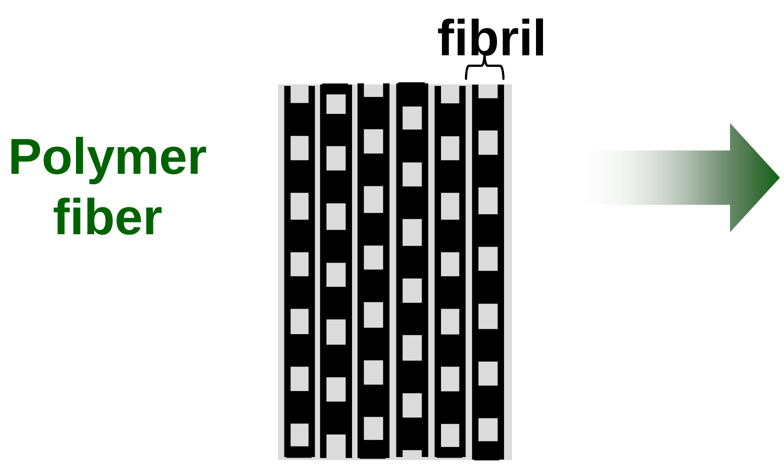
### Fractured single nanofiber

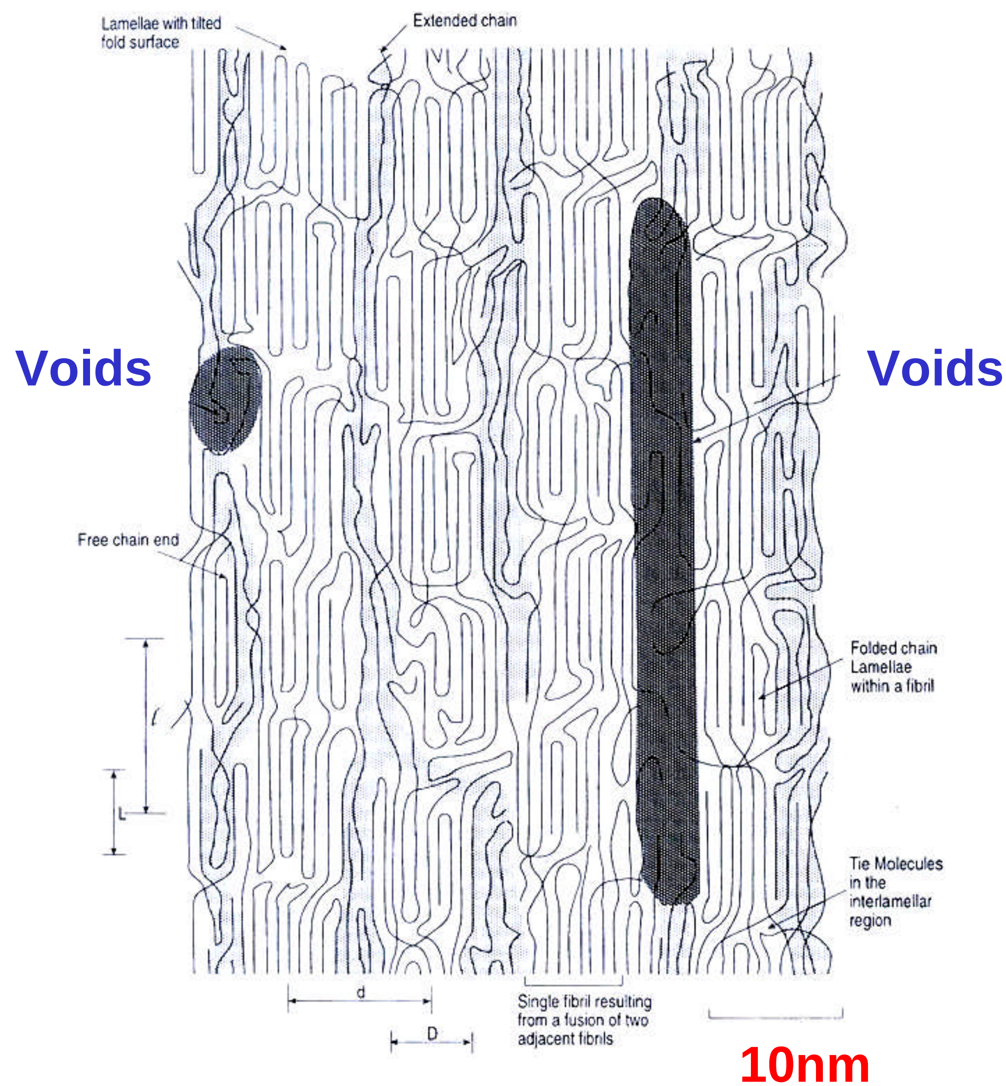


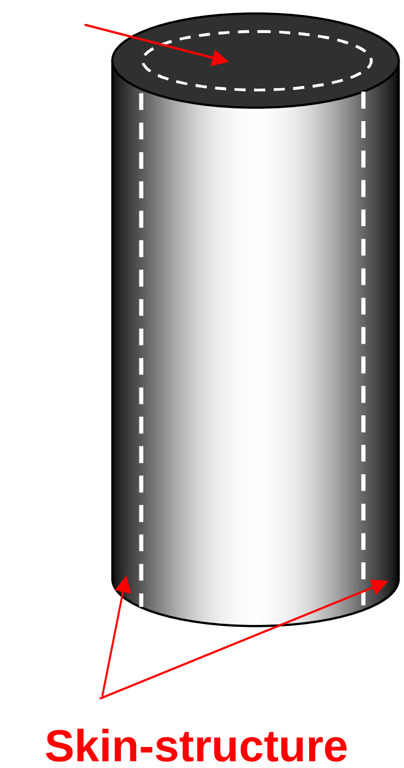
# Molecular Structure

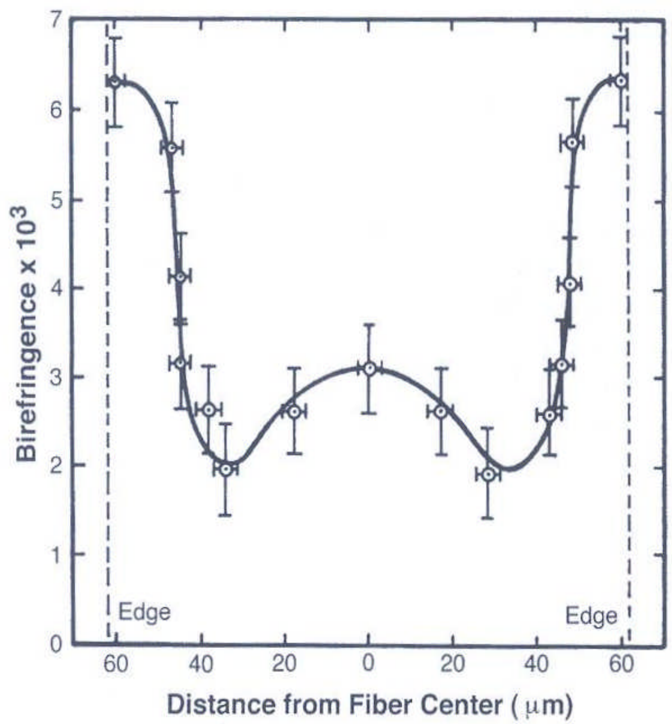
A fiber generally could be regarded to consist of crystalline regions, non-crystalline regions (often referred to as amorphous) and voids.



# Surface Structure Vs Bulk Structure




*High-density Polyethylene (HDPE)*

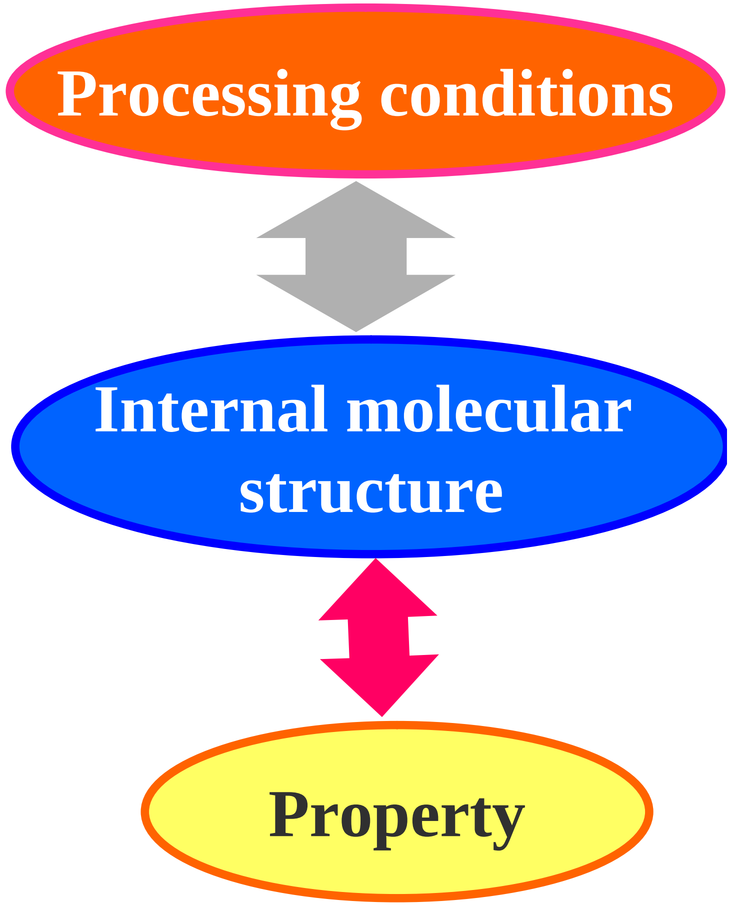
**Birefringence across the diameter of a thick HDPE filament**

**Fung et al. 1972**

| Conditions | Fiber diameter [μm] | Diameter of core-structure [μm] | Thickness of skin structure [μm] |
|---|---|---|---|
| Melt-spun fibers *(micron-fiber)* | 126 | 76 | 25 |
| Electrospun fibers *(nano-fiber)* | < 1 | ? | ? |

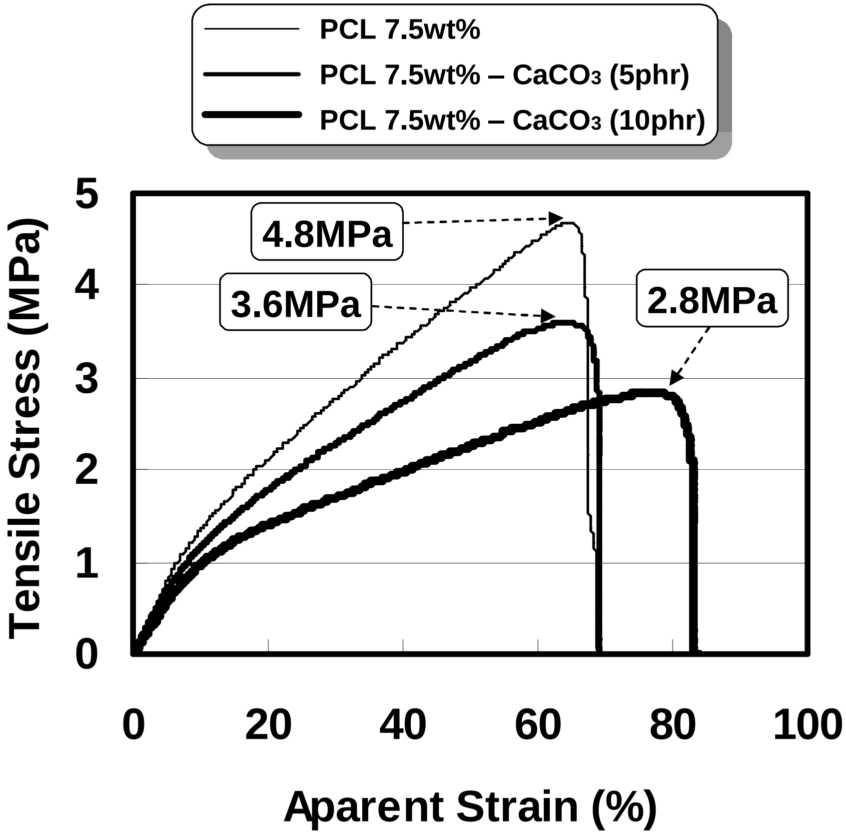
# Modeling of Nanofibers

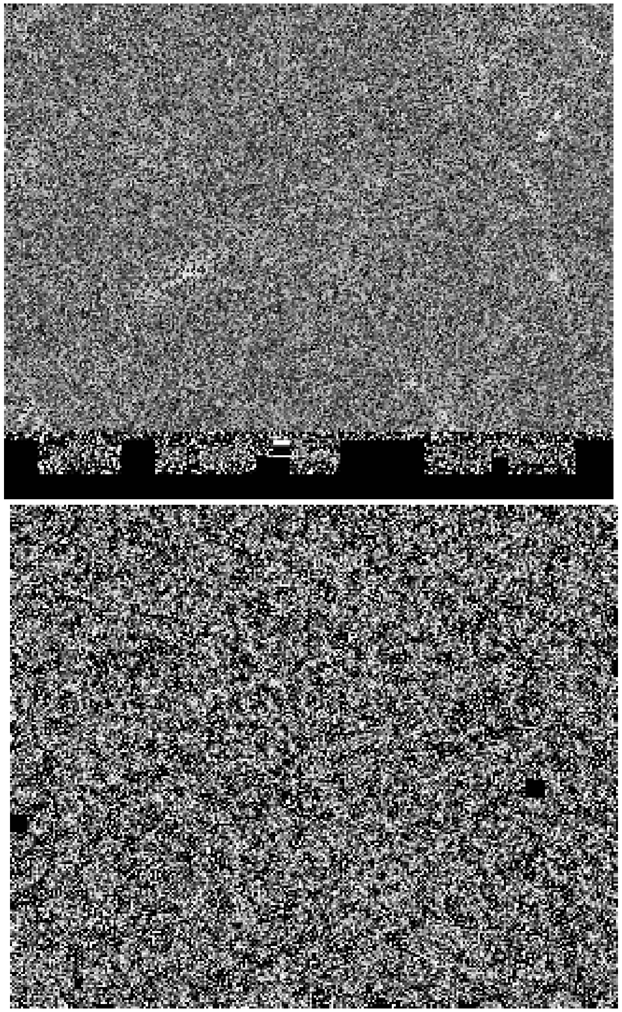
## Key Considerations




- Molecular Weight
- Percentage Crystallinity
- Orientation of Crystallites
- Porosity of Nanofibers
- Size-dependency via surface modulus

# Composite Nanofibers



SEM

PCL / CaCO3
25 / 75

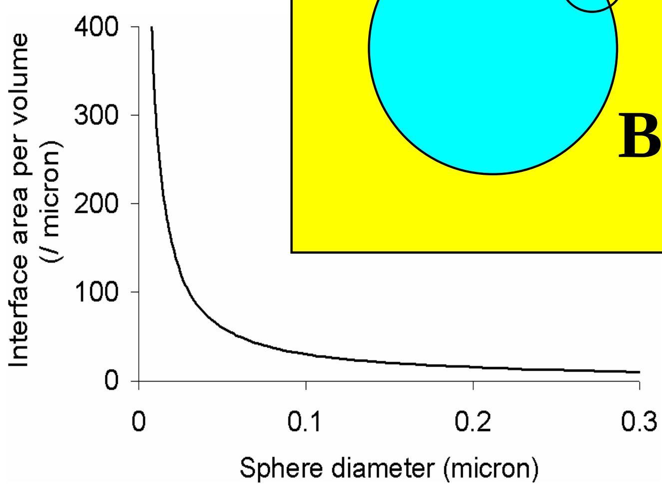
EDX

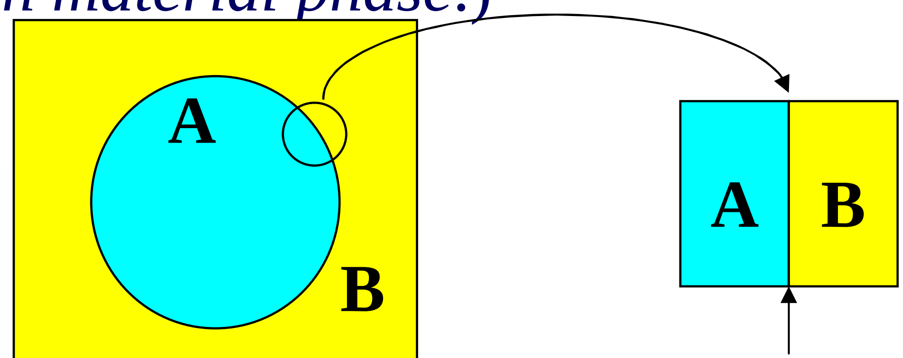
Fujihara, K., Kotaki, M. and Ramakrishna, S. (2003) Structure-Property Relationship of Electrospun Polymer/Calcium Carbonate Composite Nanofibers. Proceedings of the 8th Japan International SAMPE Symposium and Exhibition, November 18-21, Tokyo, Japan, pp. 1213-1216.

# Modeling for Composite Nanofibers

For bulk nanocomposites, volume fraction of each material phase take precedence over interface properties. (*Number of bondings between phases is negligible compared to bondings within each material phase*.)

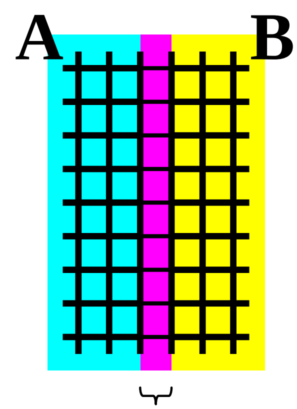
For composite nanofibers, interface properties are important. (*Number of bondings at interface is significant compared to those within each material phase*.)




**Interface**

(*surface*)

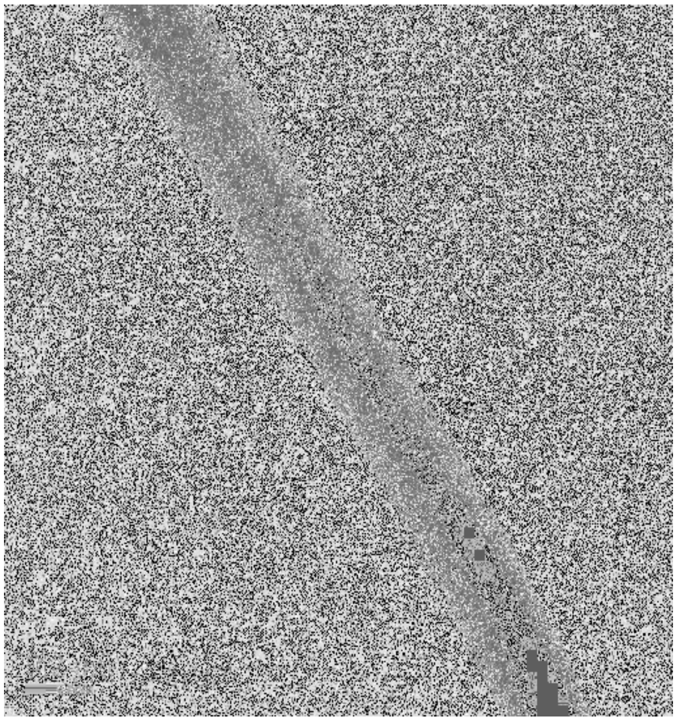


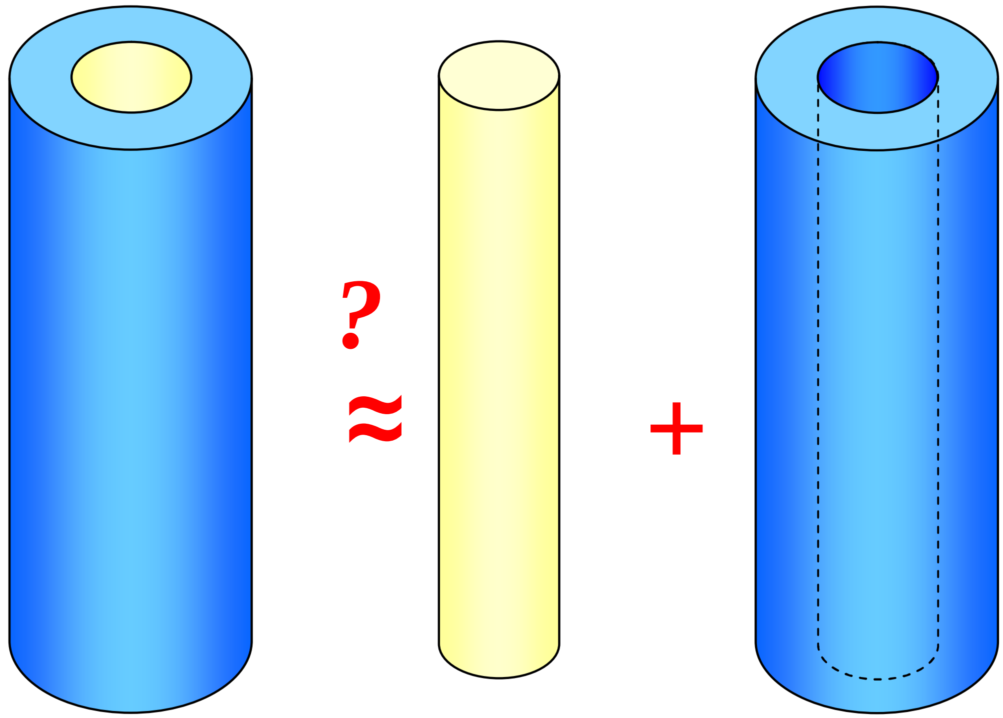

**Interphase**

**With ionic bonding**

(*volume*)

# Modeling of Shell-Core Nanofibers



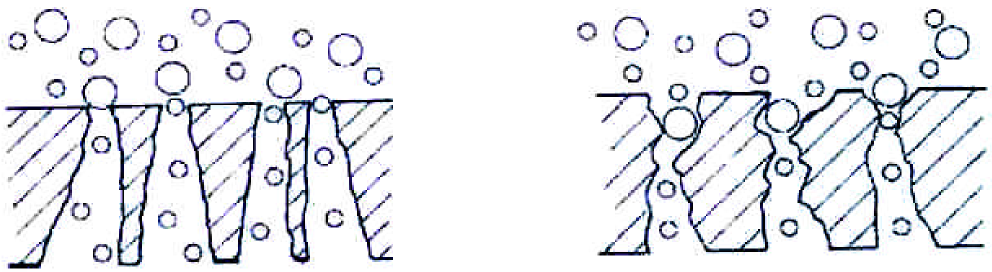


# Outline

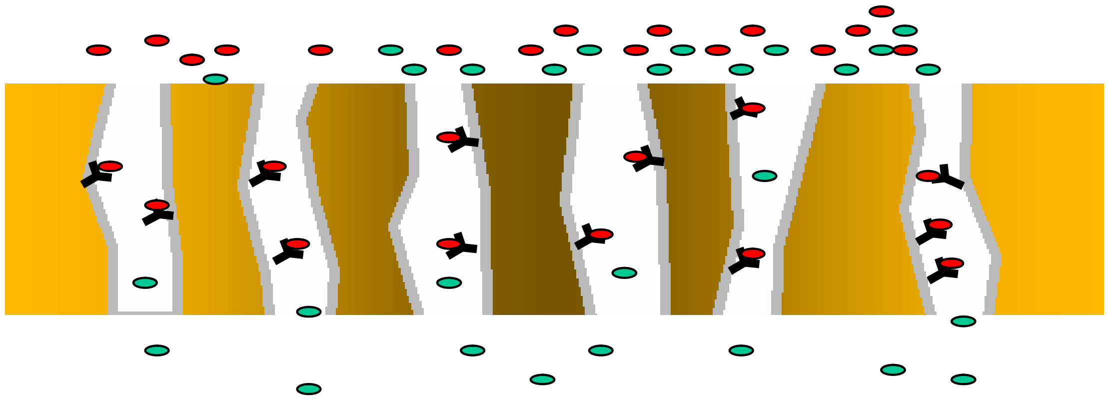
- Motivation for Nanofibers
- Quest for Polymer Nanofibers
- Electrospinning - State of the Art
- Affinity Membranes
- Tissue Engineering

# What is affinity membrane?

- In affinity separation, the separation is not largely due to size but rather on the selectivity of the matrix to 'capture' molecules.
- Great potential in biological systems for purifying/separating proteins or other biomolecules from biological fluids



Size exclusion membrane



Affinity membrane

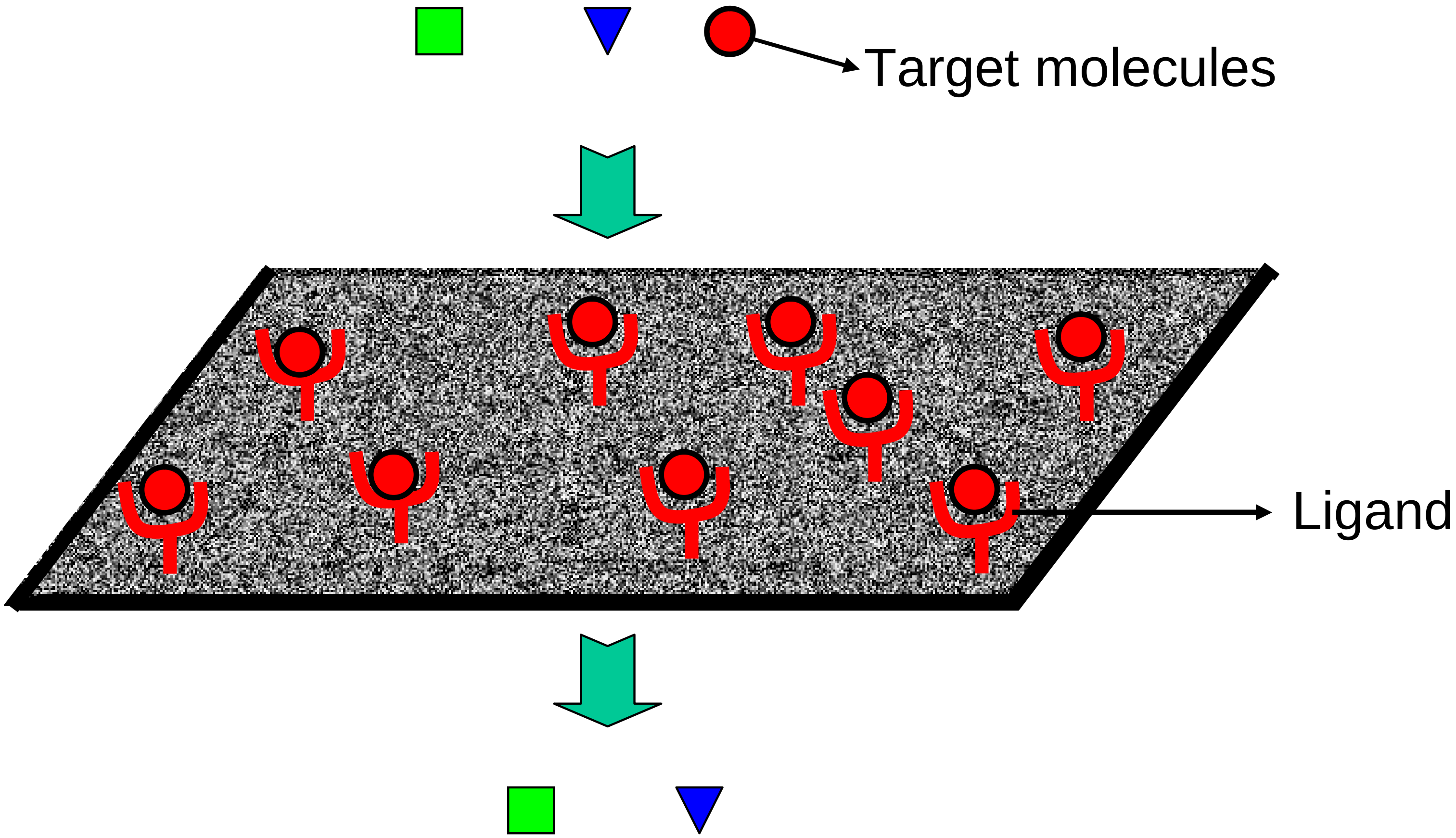
# Important aspect of Affinity Separation

NUS
National University of Singapore

## Large surface area to volume ratio

- Ability to immobilize a high density of ligands
- provide a large area for the contacting between the ligand and the target molecules, which facilitate the capturing process.
- Directly affects the performance (efficiency)

# Functionalized electrospun nanofiber as affinity membrane



# Affinity Membranes

***Can the electrospun nanofiber mesh be surface functionalized and applied as affinity membrane?***

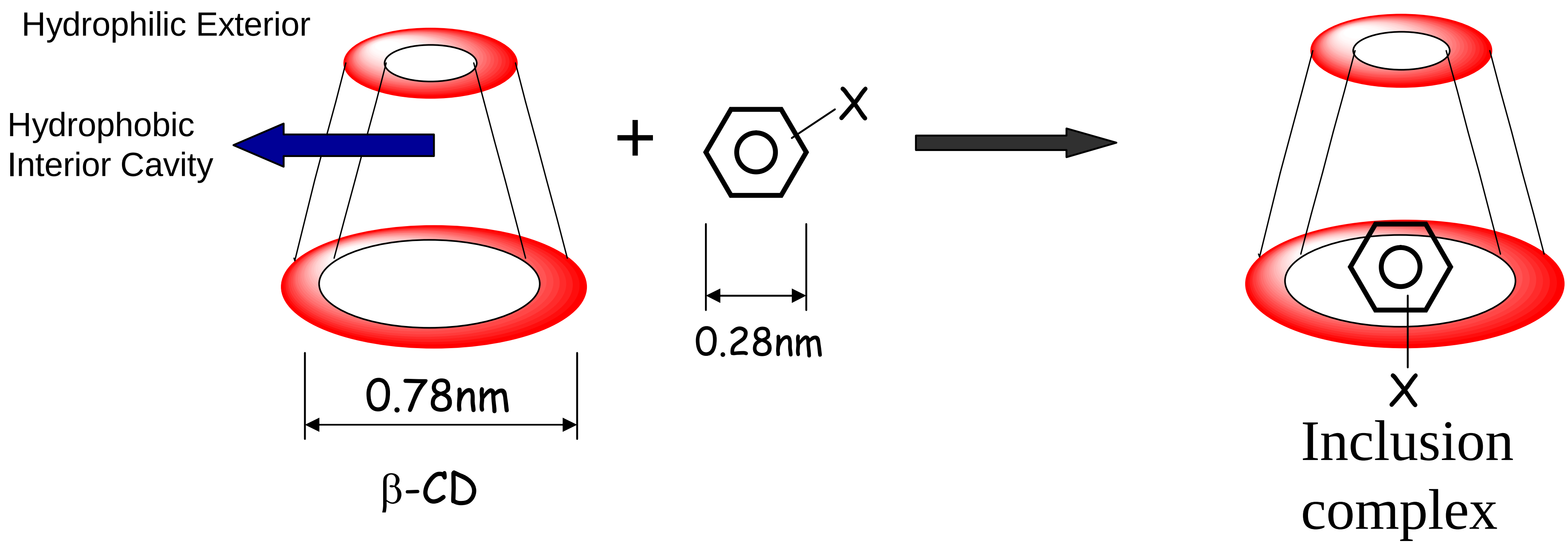
1. CD functionalized polymer nanofiber for organic waste removal

2. S3Δ immobilization on nanofiber surface for LPS (endotoxin) removal

3. BSA functionalized polymer nanofiber for bilirubin removal

1.CD functionalized polymer nanofiber for organic waste removal

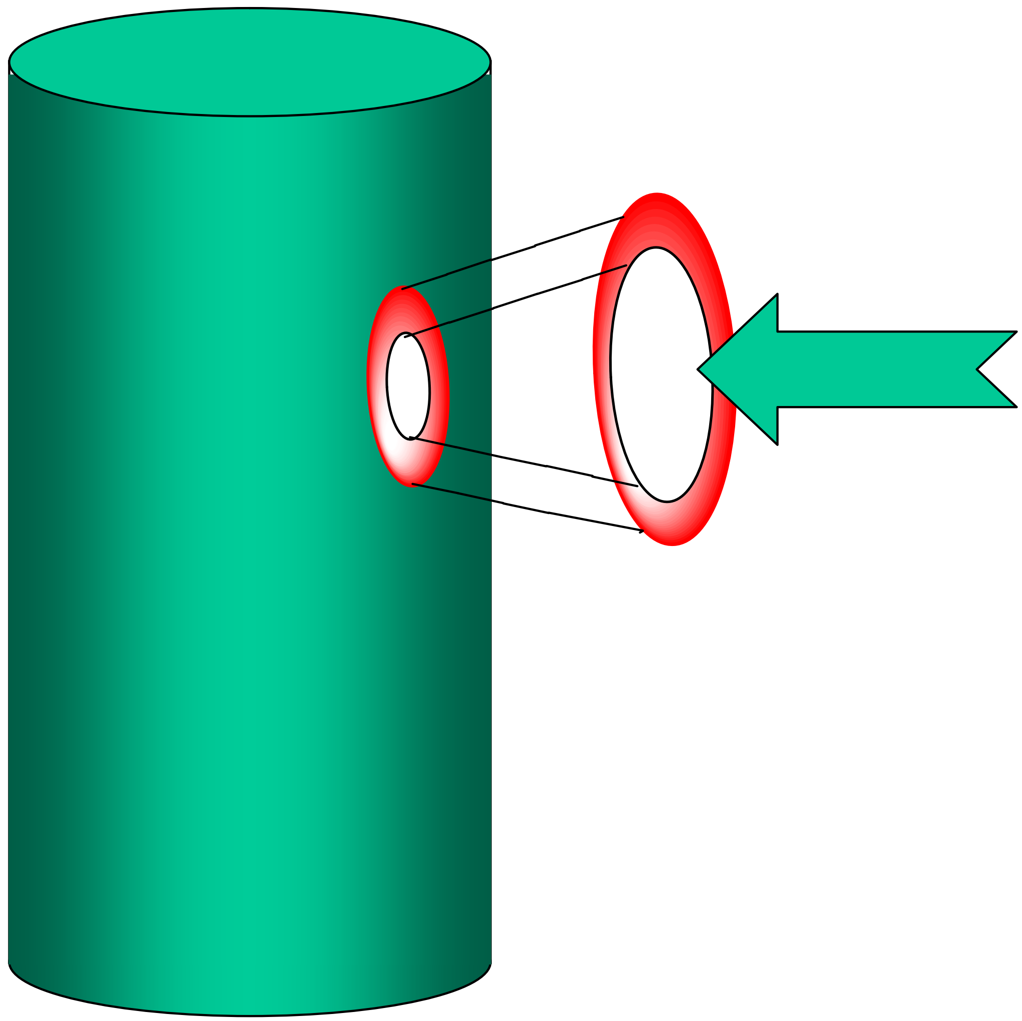
# Key structural feature and application of CD

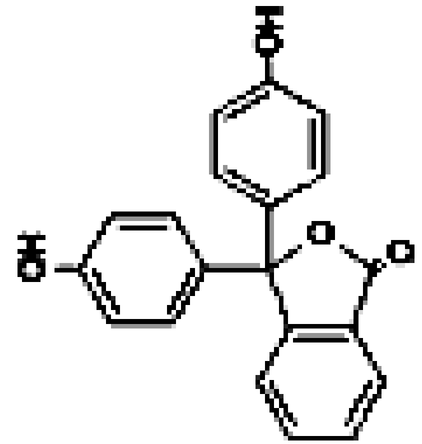



**Application:**

- Solubilization of various water-insoluble compounds
- Ability to discriminate between positional isomers, functional groups, enantiomers

# Affinity Membranes

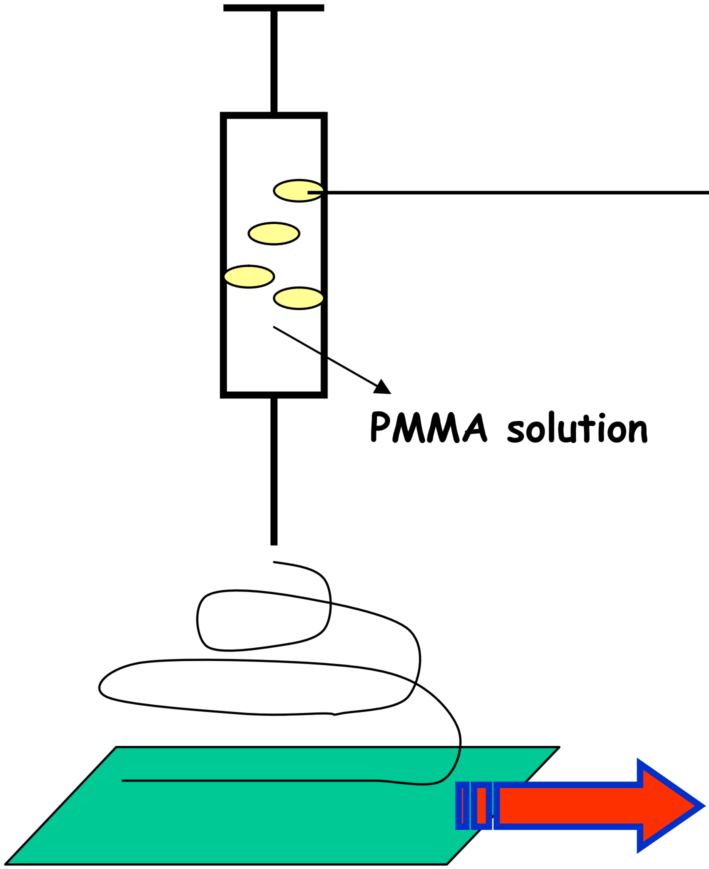


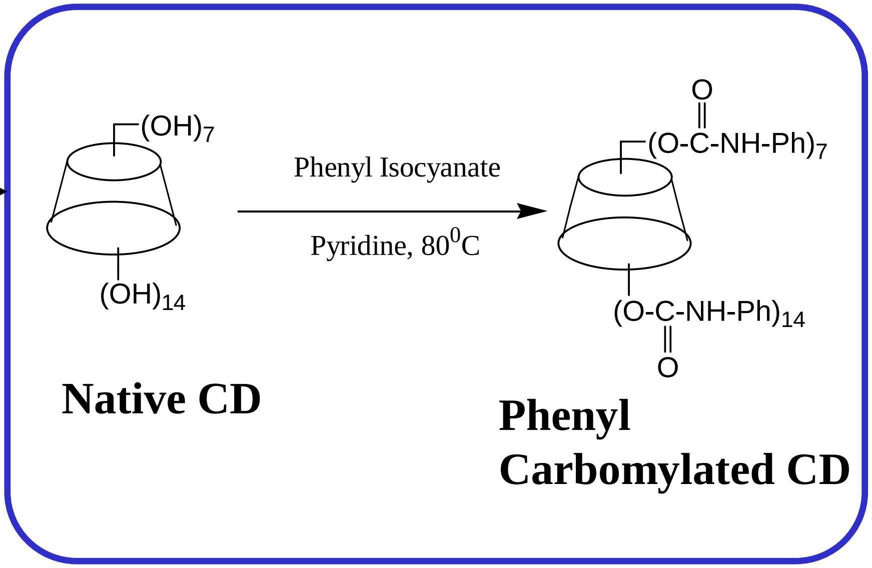


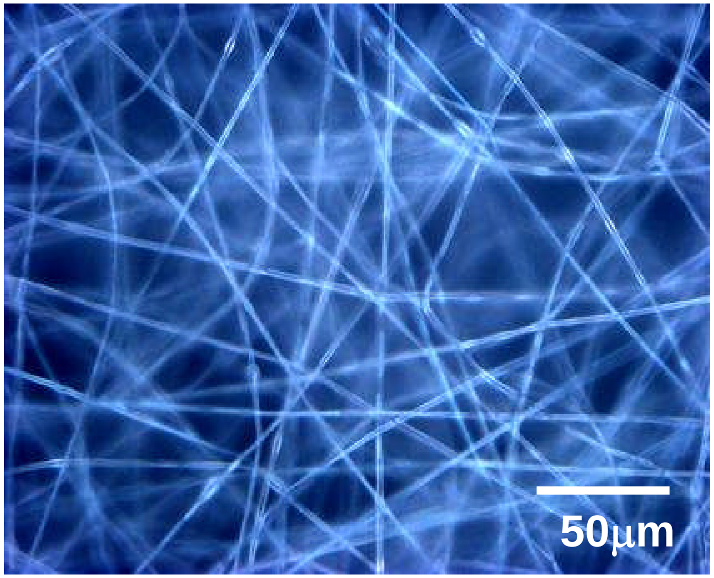
Phenolphthalein (PHP)

Electrospun polymer nanofiber

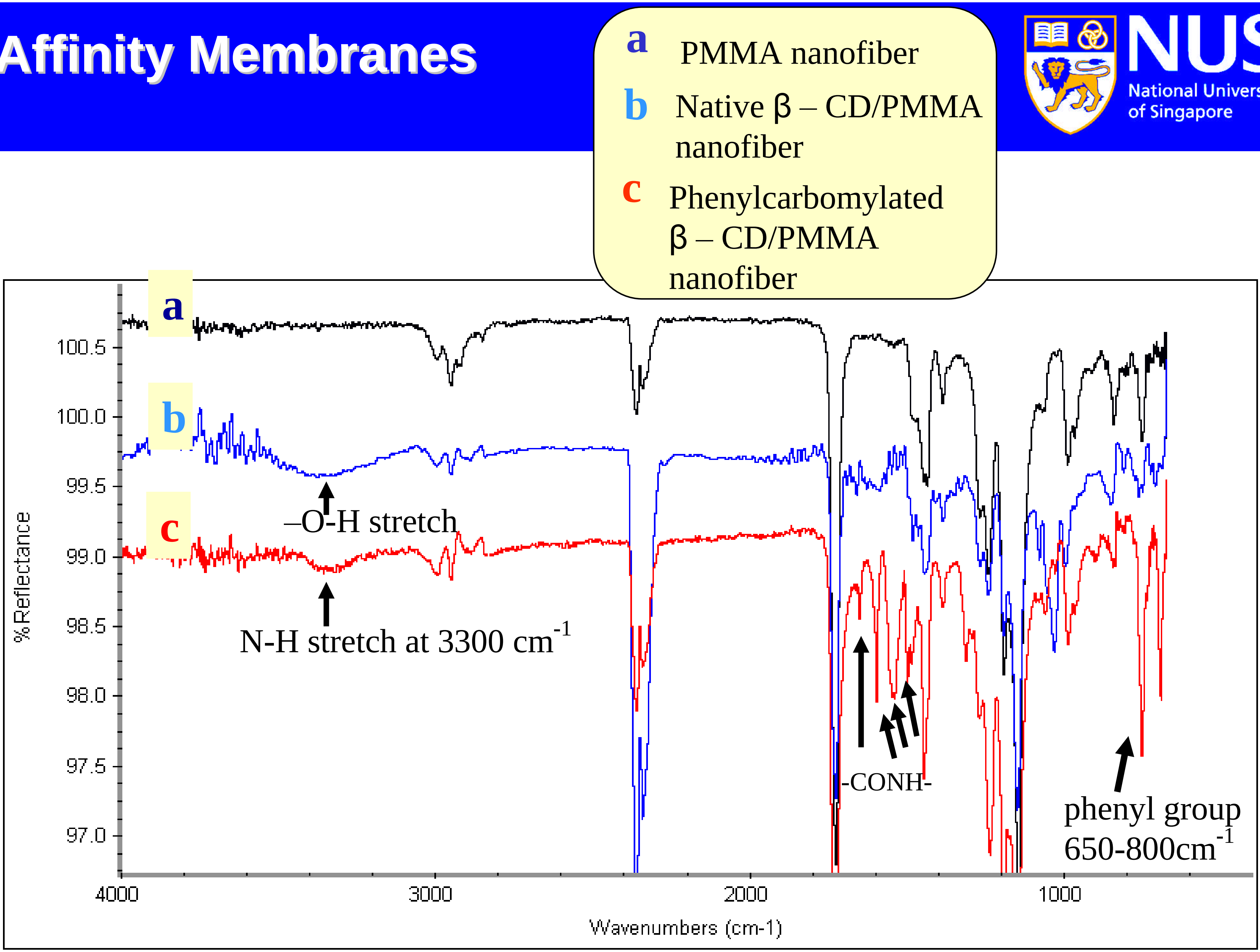
# Affinity Membranes



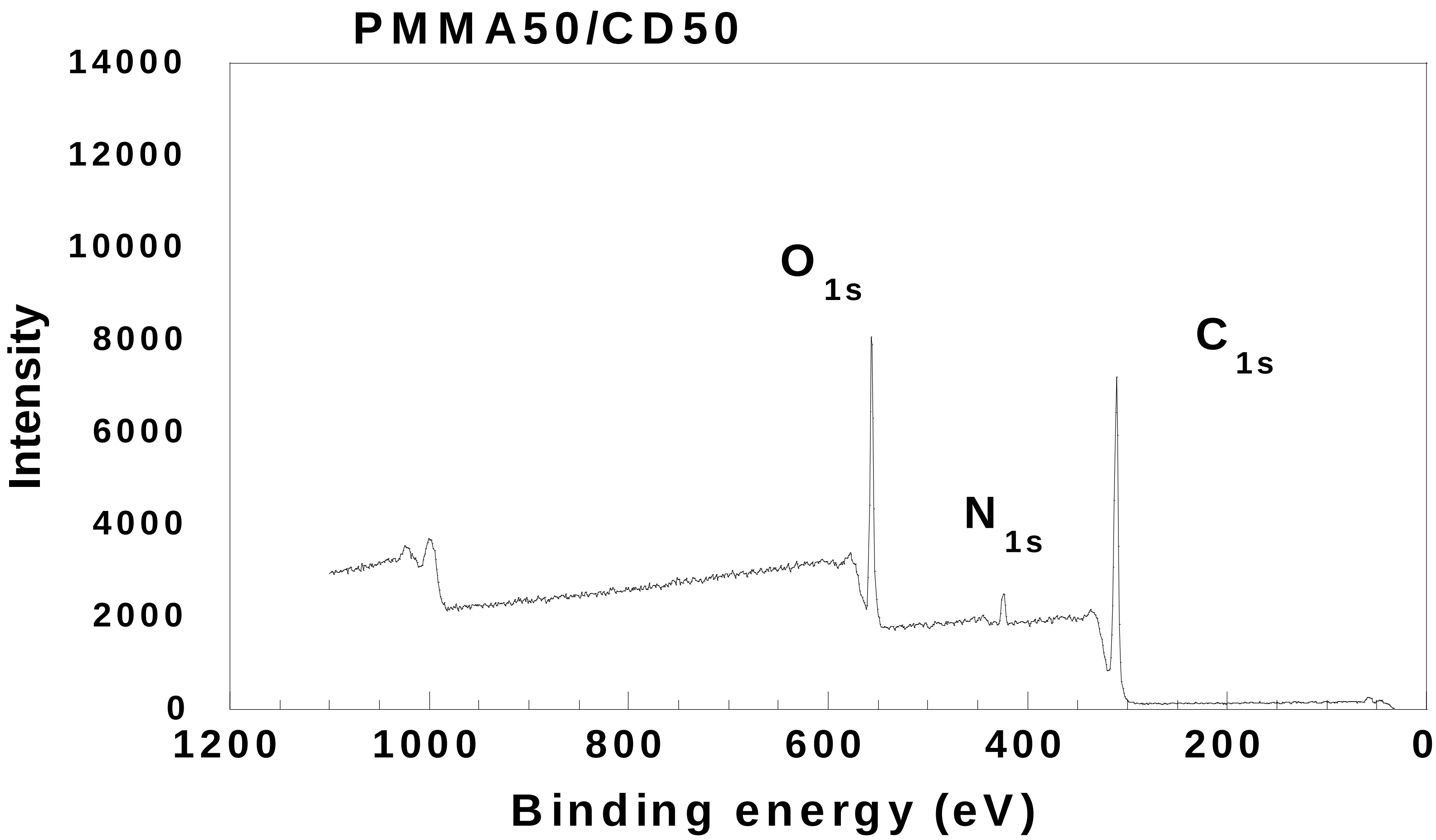



# Affinity Membranes

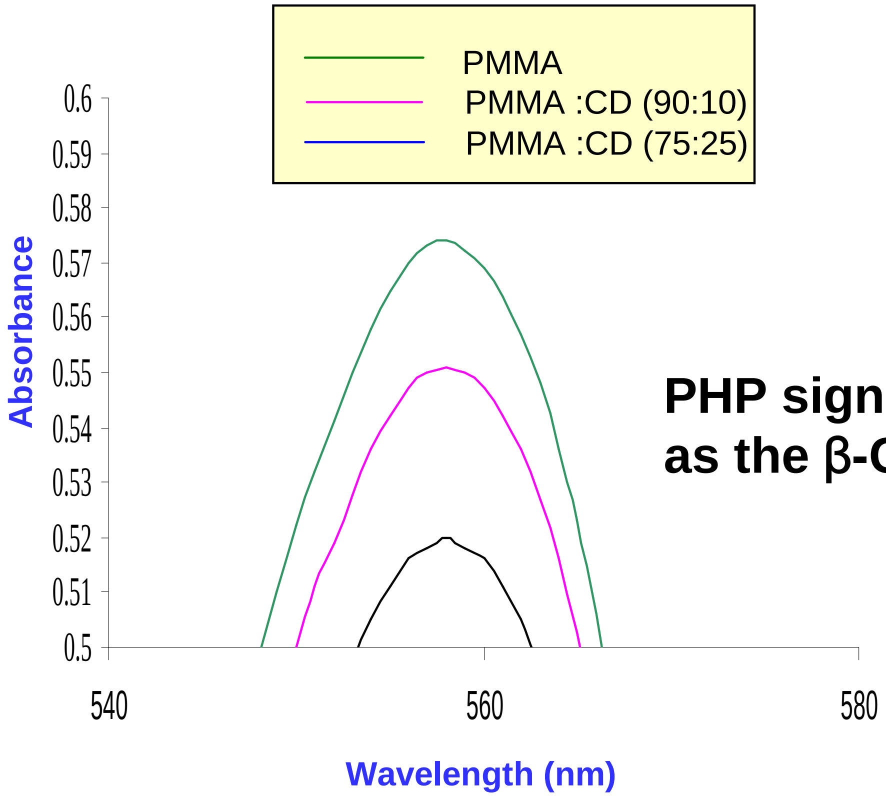


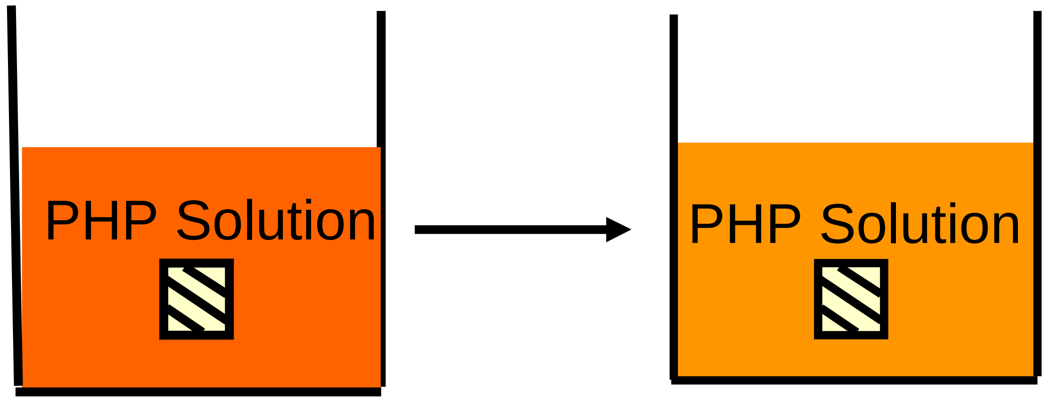
# Affinity Membranes



# Affinity Membranes

## UV/VIS Absorbance of PHP



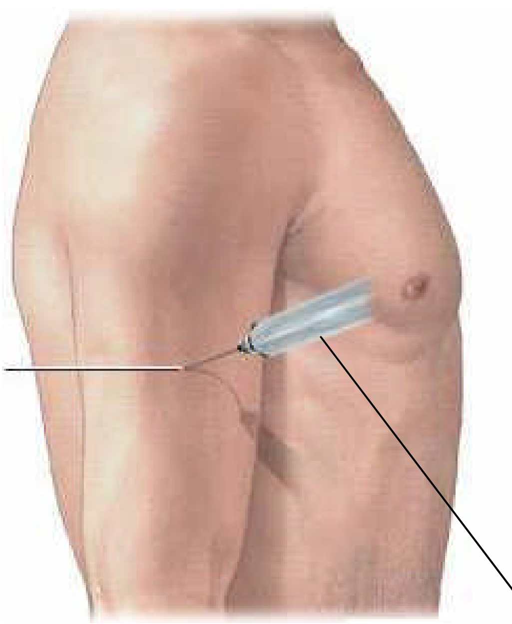




**PHP signal decreases as the β-CD concentration increases.**

2. S3Δ immobilization on nanofiber surface for LPS (endotoxin) removal

# Affinity Membranes

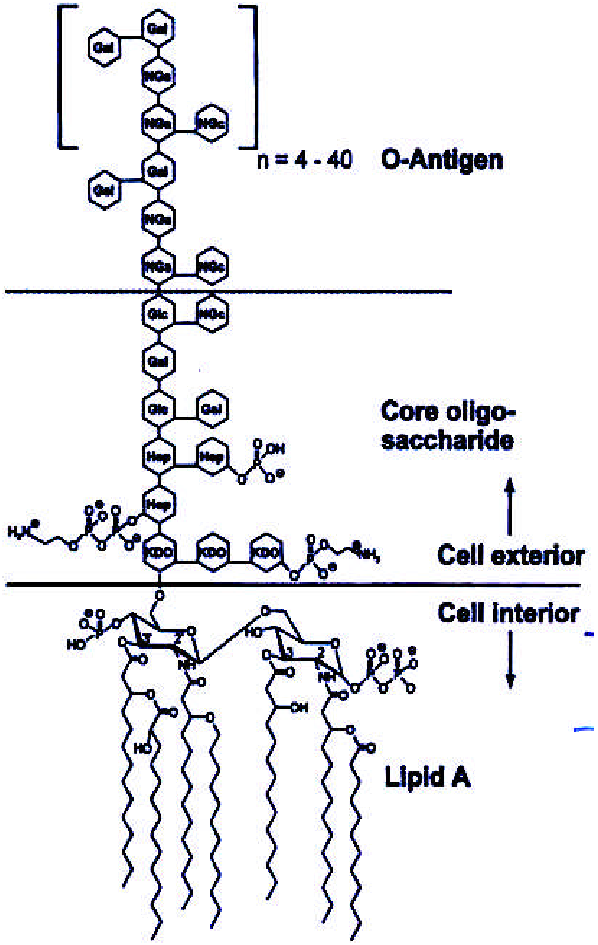
Endotoxins (lipopolysaccharide - LPS) is a poisonous substance liberated by gram-negative bacteria. Sever septic shock caused by endotoxin during bacterial infections **is responsible of over 100,000 death in US alone.**



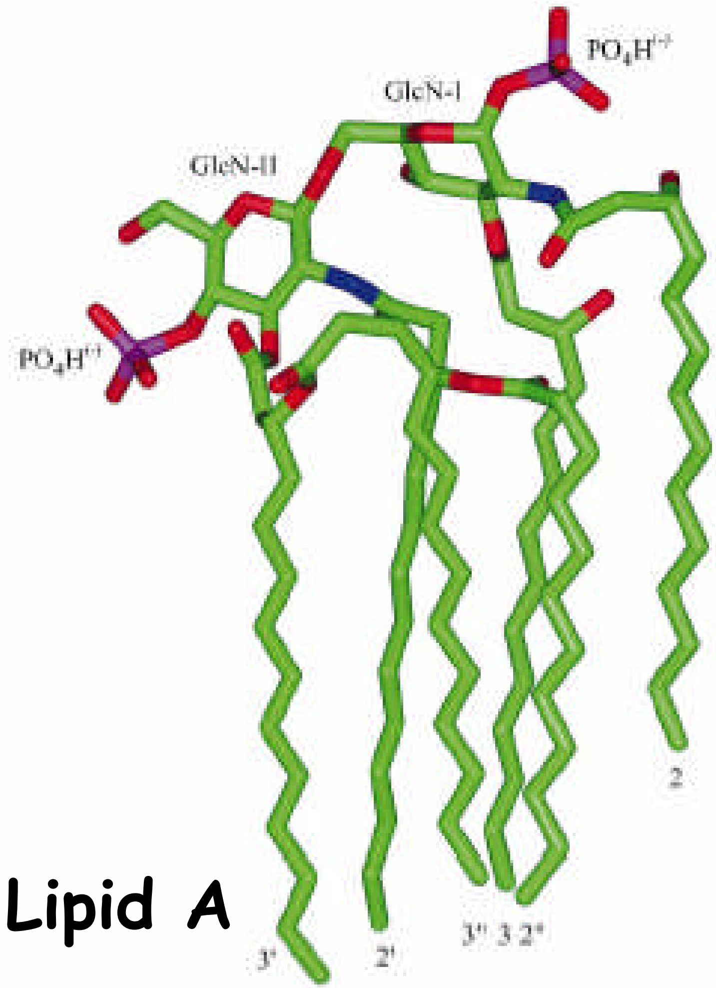
Must be endotoxin free!

LPS is a frequent contamination in many heath-care injection products derived from bioprocesses.

# Affinity Membranes




LPS




**Lipid A**

**Toxic portion of LPS, a highly conserved bioactive center of LPS**

*V. Frecer et al. Biochimica et Biophysica Acta 1466 (2000) 87-104*

NUS National University of Singapore

# S3Δ peptide

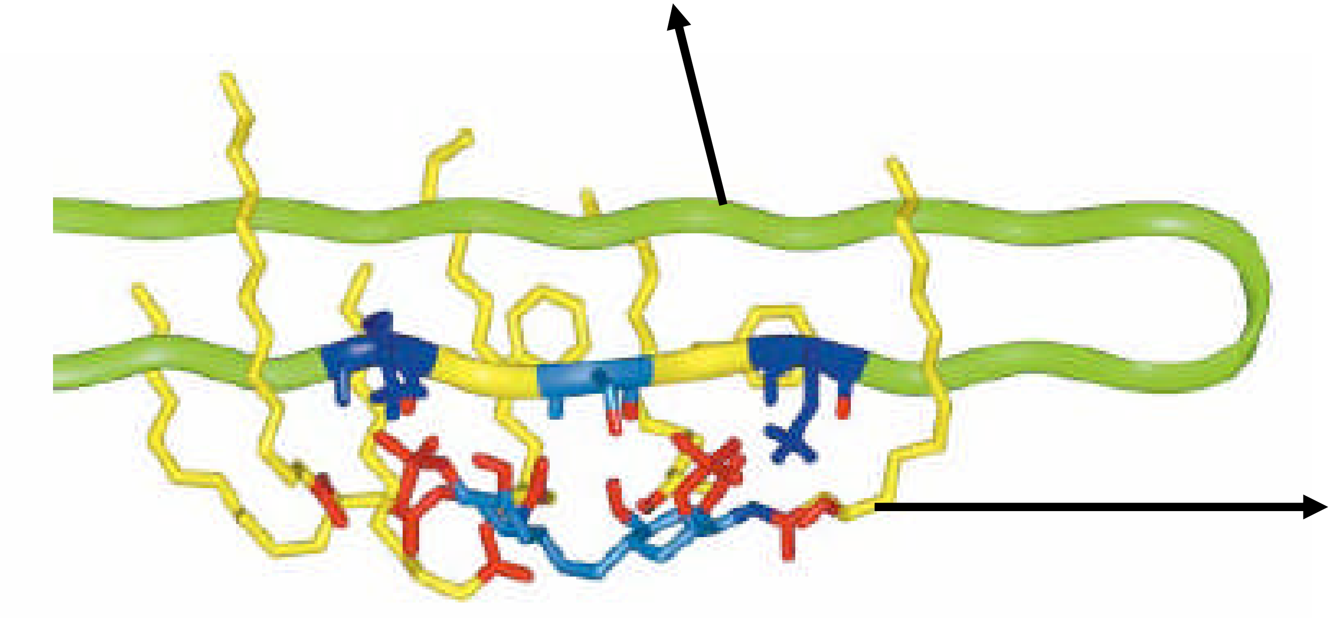
**($NH_2$-HAEHKVKIKVKQKYGQFPQGTEVTYTCSGNYFLM-COOH)**

S3△ contains 34 amino acids and corresponds to a highly specific LPS binding sushi domain (268-301) in factor C, a well known LPS binding protein found in blood of horseshoe crab.

S3△ is able to bind with the toxic portion of LPS (Lipid A) with highly specific affinity constant ($k_a$= $6.5\times10^{2}$ $M^{-1}S^{-1}$)

# Affinity Membranes

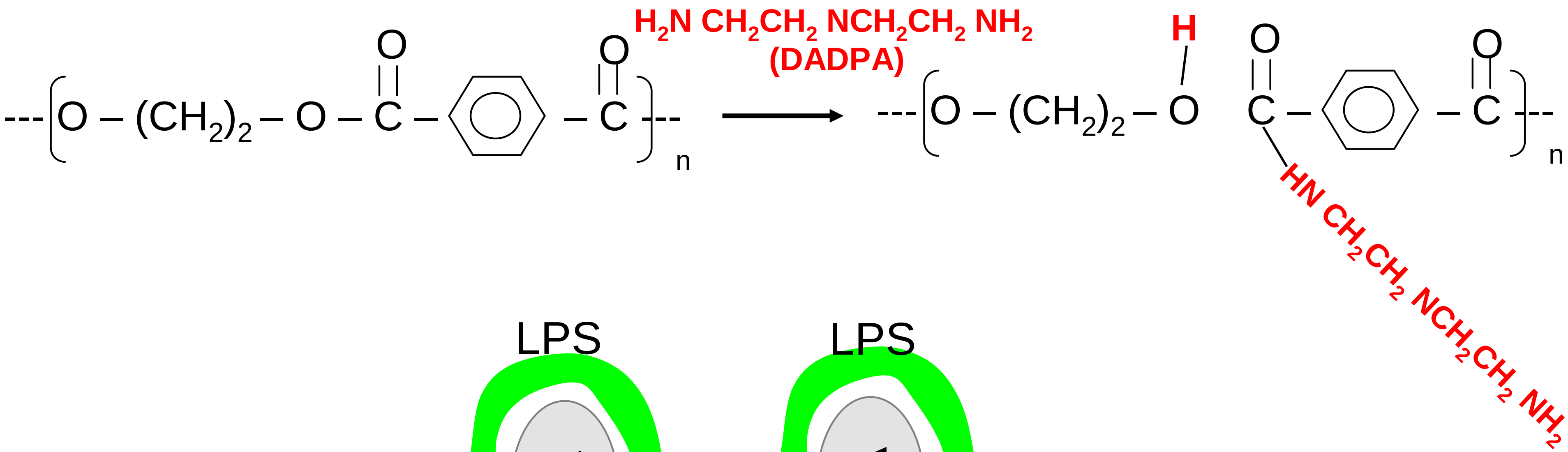
NH2-HAEHKVKIKVKQKYGQFPQGTEVTYTCSGNYFLM-COOH

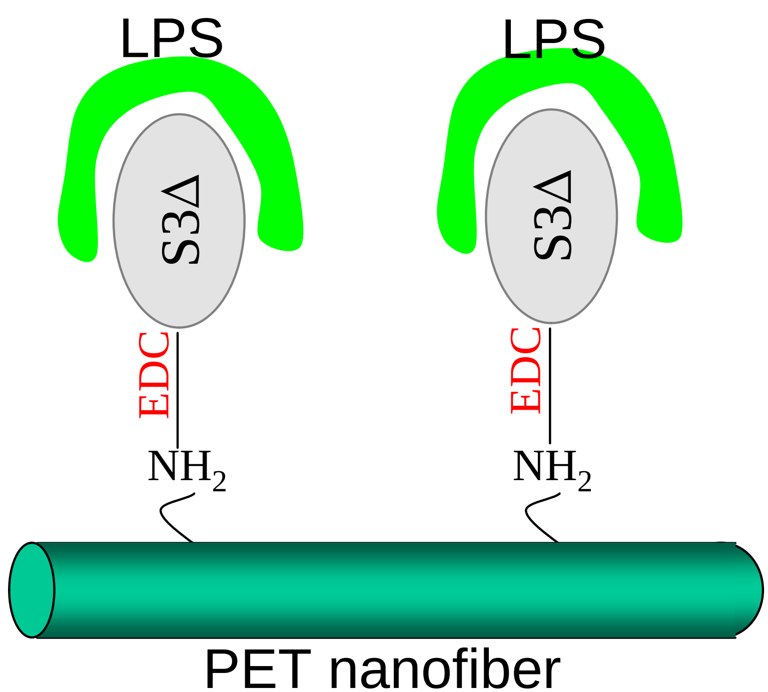



A proposed model of combination of the S3Δ peptide and Lipid A. *V.Frecer et al. Eur J Biochem 267*

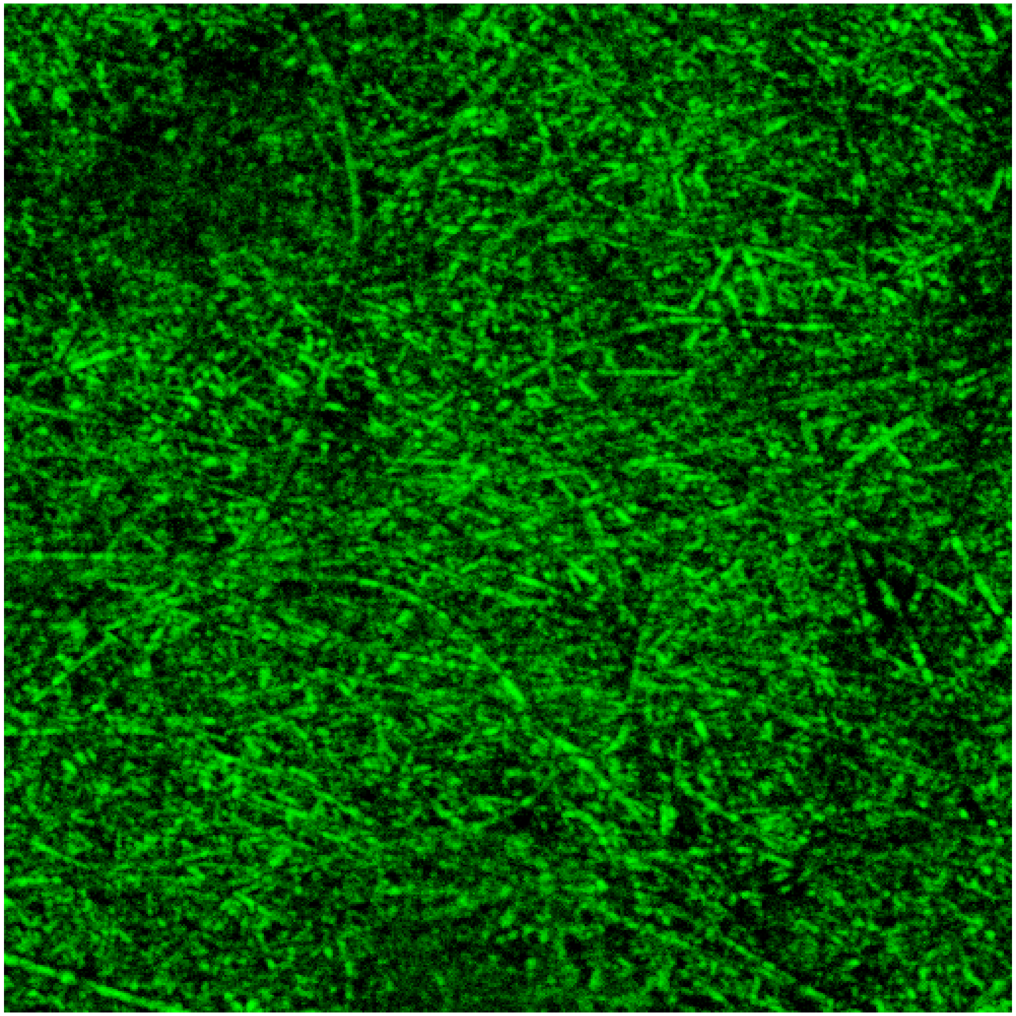
# Affinity Membranes

NUS National University of Singapore



# Affinity Membranes

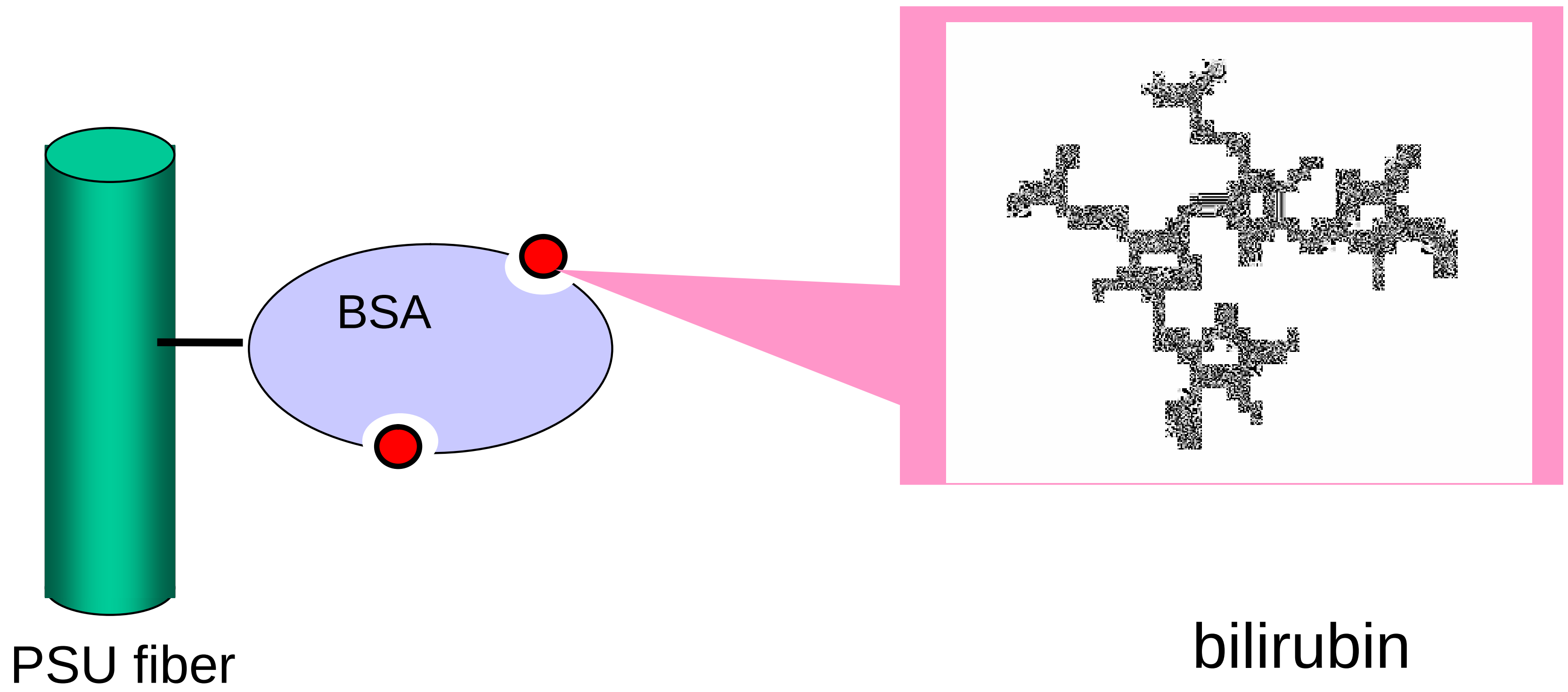


FITC-LPS captured by the S3Δ peptide functionalized PET nanofiber

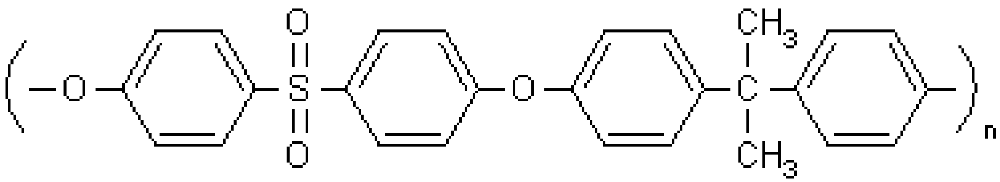
3. Bovine serum albumin (BSA) immobilization on the PSU nanofiber for bilirubin removal

# Affinity Membranes

Bilirubin is a metabolic waste product in human blood and has higher concentrations in blood of patients suffering from hyperbilirubinemia. The excessive bilirubin must be removed especially for the baby, of whom the brain development can be affected.

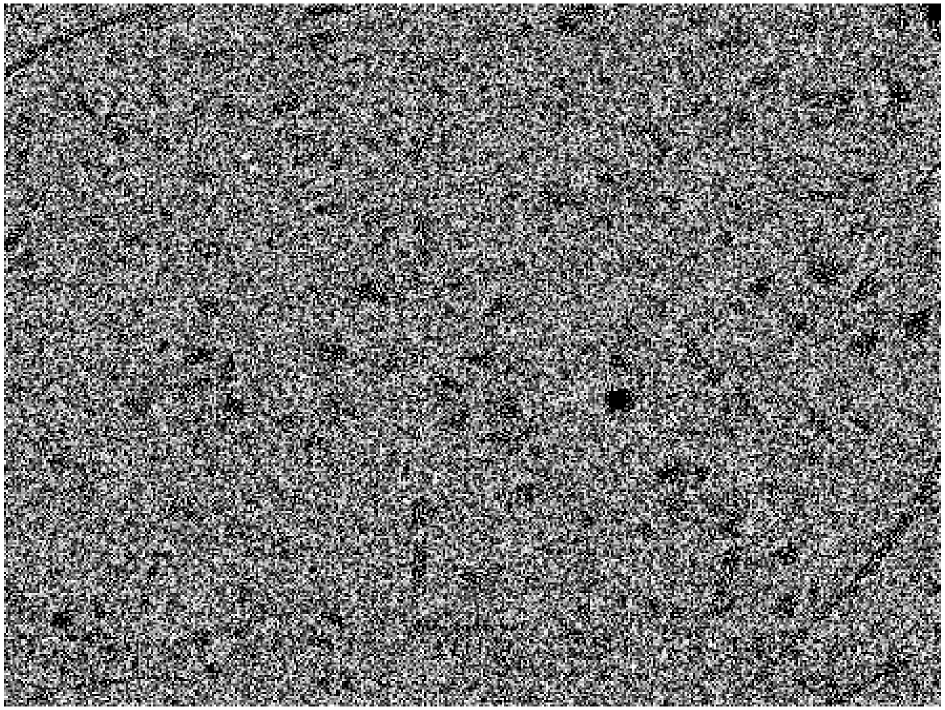


# Affinity Membranes

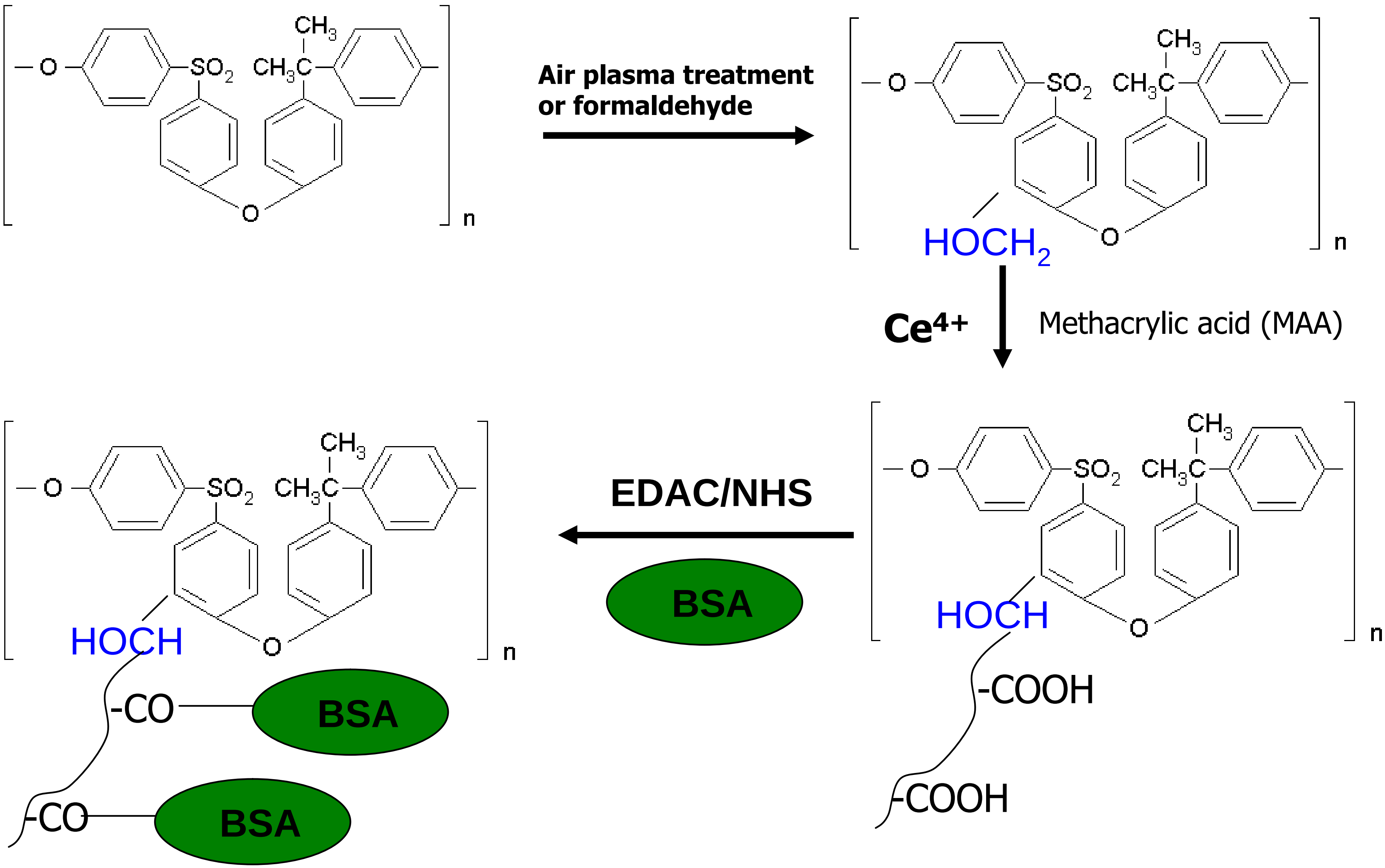


Polysulfone

Polysulfone/Pyridine solution, 30 wt%.



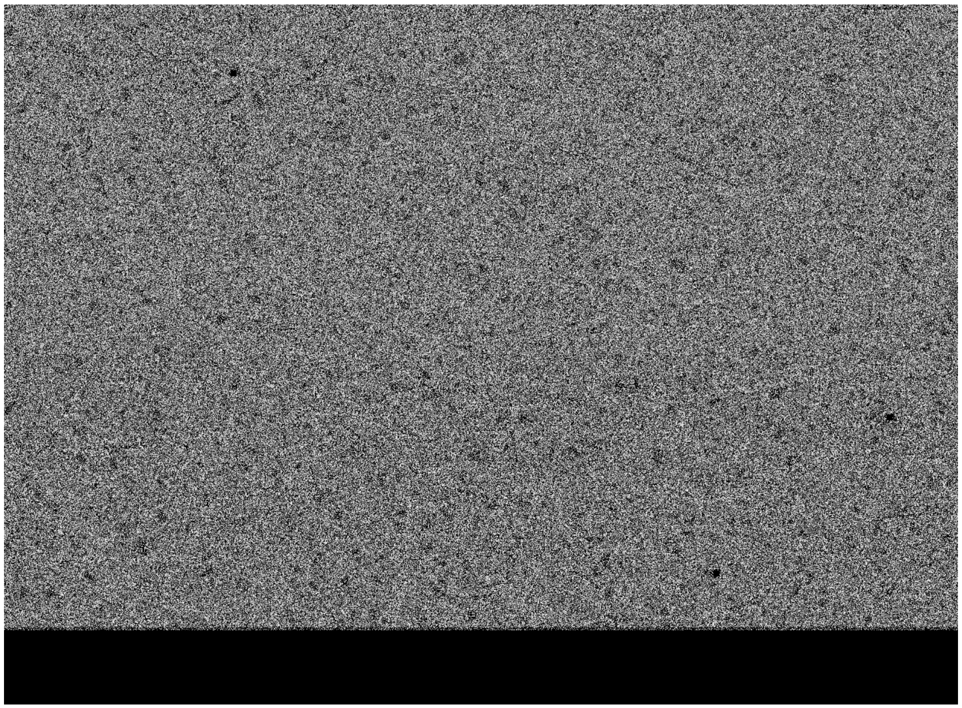
# Affinity Membranes

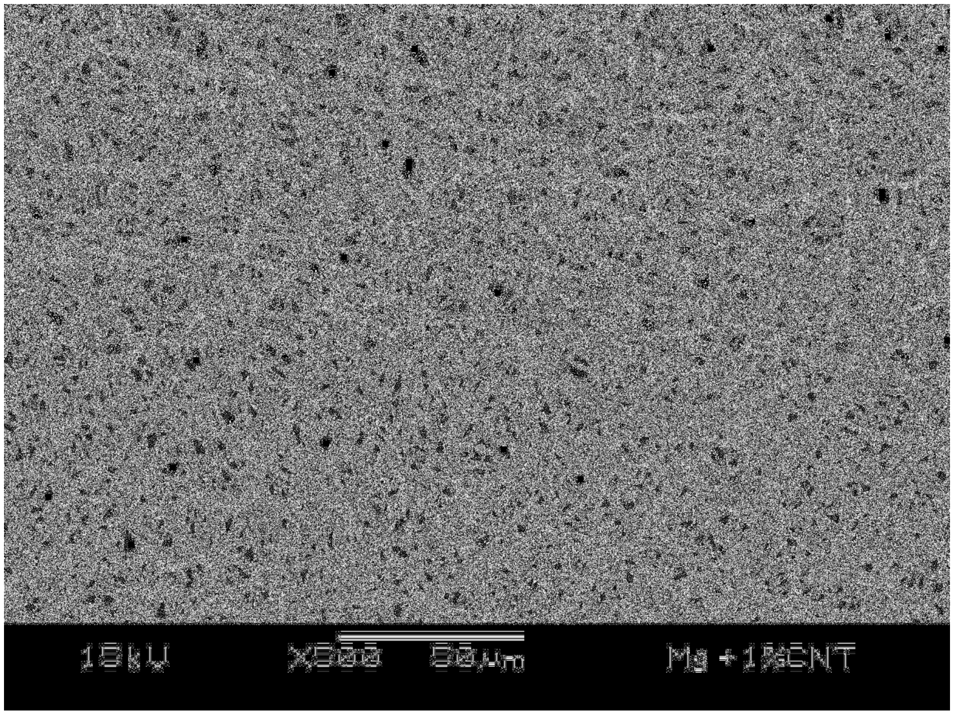



Reaction Scheme

# Affinity Membranes

NUS
National University of Singapore



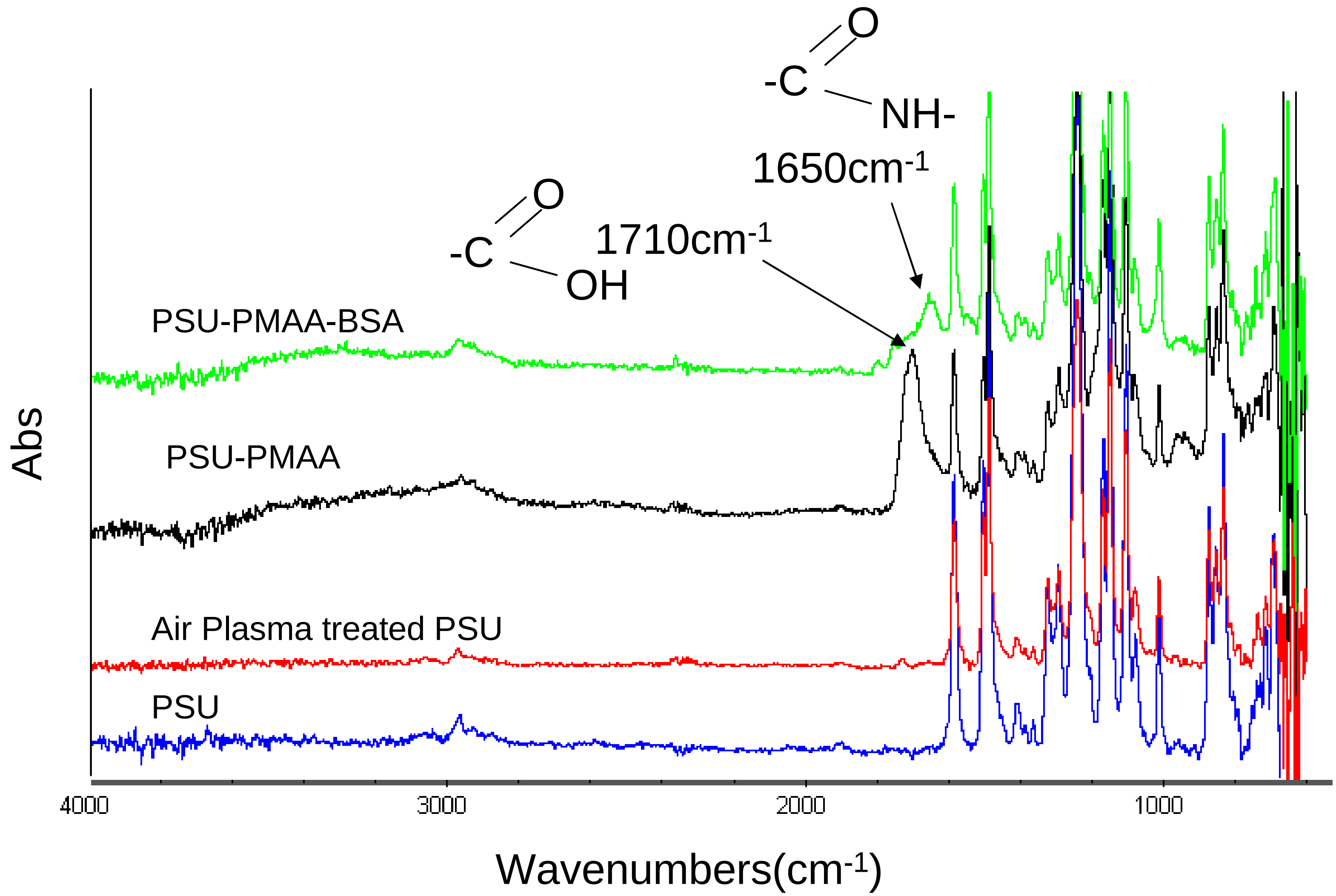



**Plasma treated PSU**

**PSU-PMAA (grafting time is 2h)**

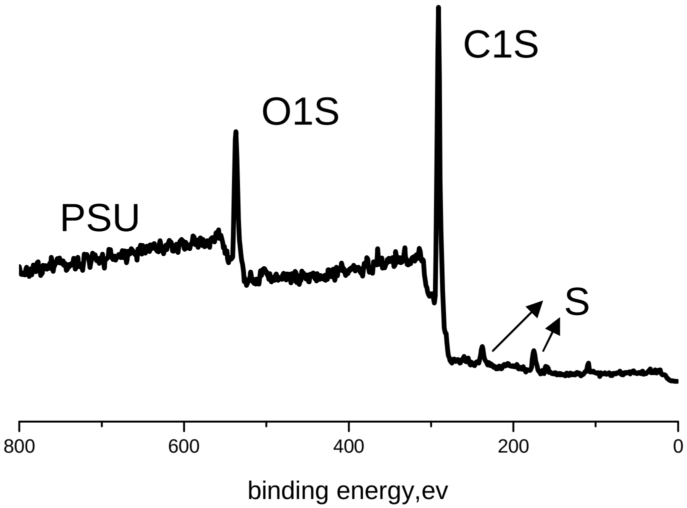
Morphology Change

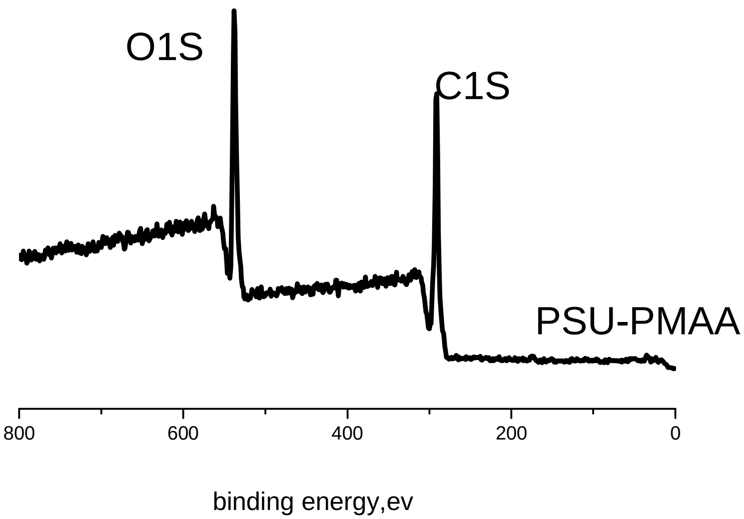
# Affinity Membranes

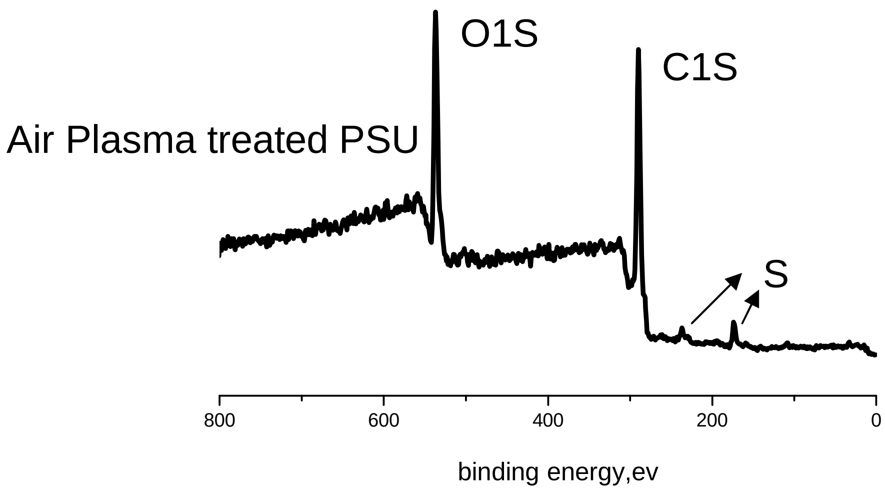


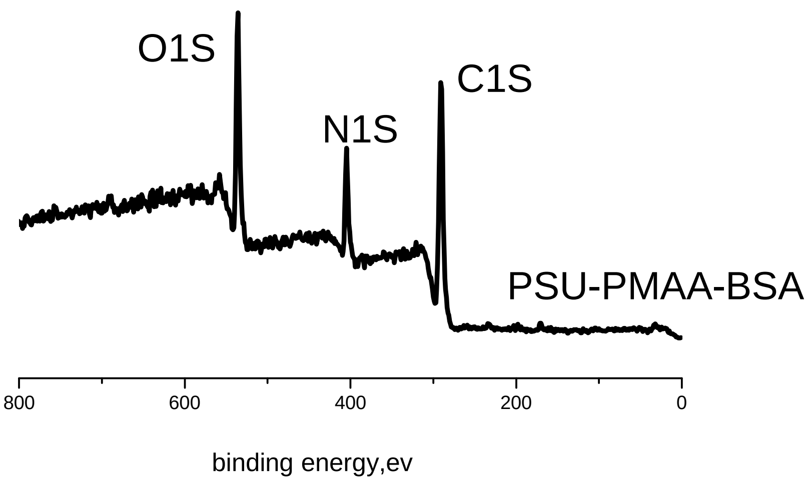

ATR Spectra of the PSU fiber mesh

# Affinity Membranes



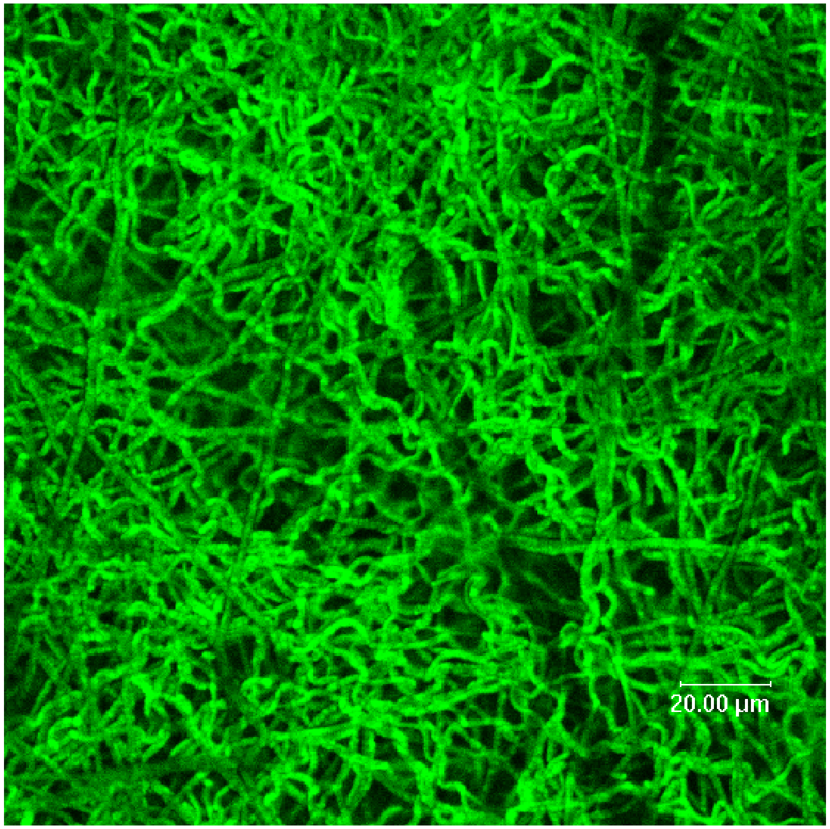



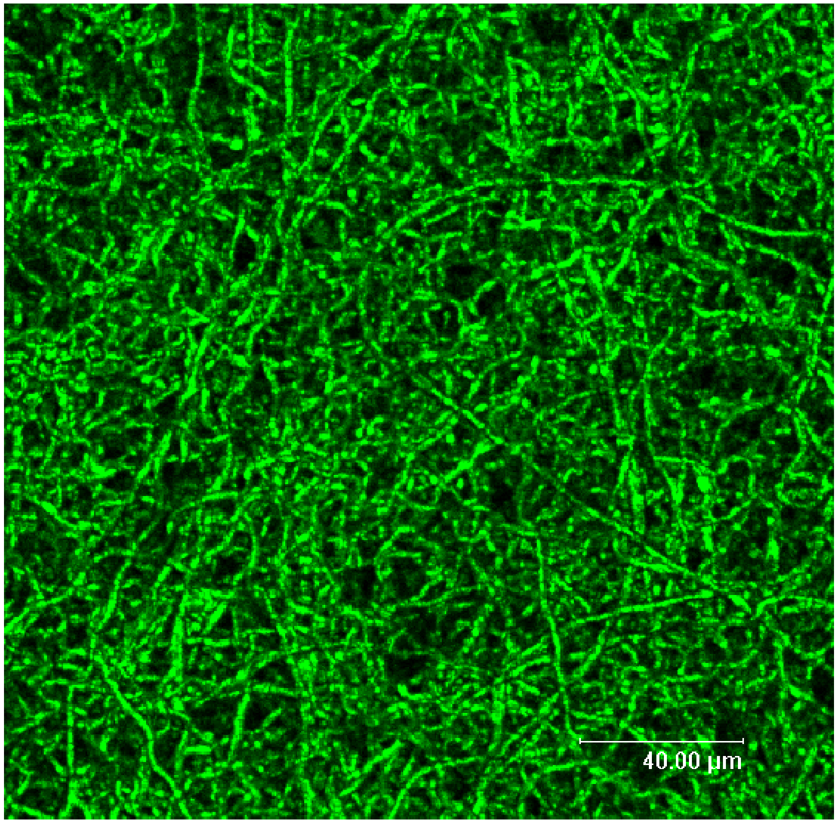
# Affinity Membranes

BSA capacity: 15μg/mg

**Bilirubin capturing ability: 0.12 μg/mg**




**FITC-BSA grafted onto the PSU fiber mat**




**Confocal image of PSU-BSA-Bilirubin**

# Affinity Membrane - Conclusions

***Can the electrospun nanofiber mesh be surface functionalized and applied as affinity membrane?***

Three kinds of ligands have been introduced onto electrospun nanofiber surface. The functionalized nanofiber showed ability to capture target molecules.

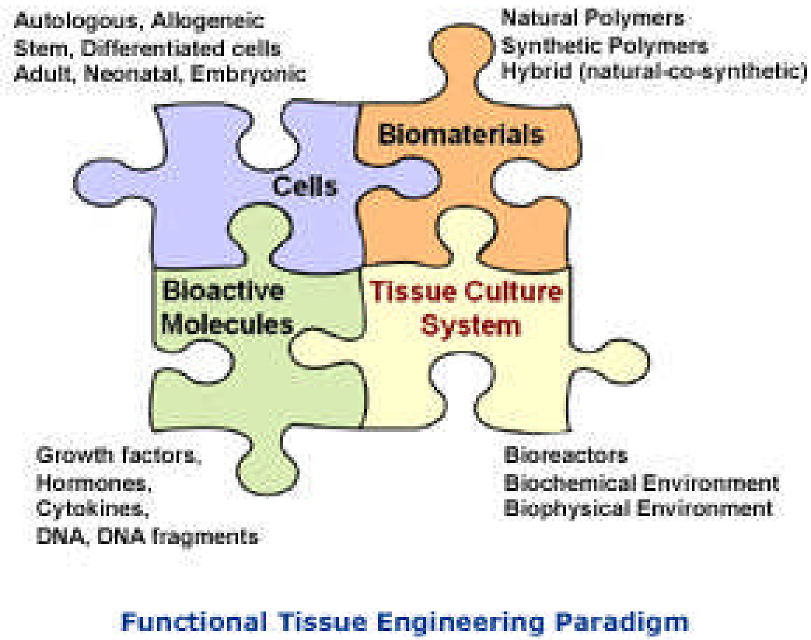
# Affinity Membrane - Future Work

- Optimization of the reaction parameters for conventional (UV induced, Ce4+ induced) surface grafting polymerization on polymer nanofiber surfaces
- Surface modification of polymer nanofibers using living radical grafting polymerization techniques (RAFT, ATRP)
- Designing of a filter setup suitable for the electrospun nanofibrous membrane and for the separation efficiency test.
- Characterization of the nanofibrous membrane’s transportation properties using the newly designed filter.
- Separation efficiency test of the nanofibrous affinity membrane using the newly designed filter.
- Development of a theoretical model for the analysis of the hydrodynamic situation, mass transfer, reaction or capturing kinetics in the nanofibrous membrane.

# Outline

- Motivation for Nanofibers
- Quest for Polymer Nanofibers
- Electrospinning - State of the Art
- Affinity Membranes
- Tissue Engineering

# Tissue Engineering



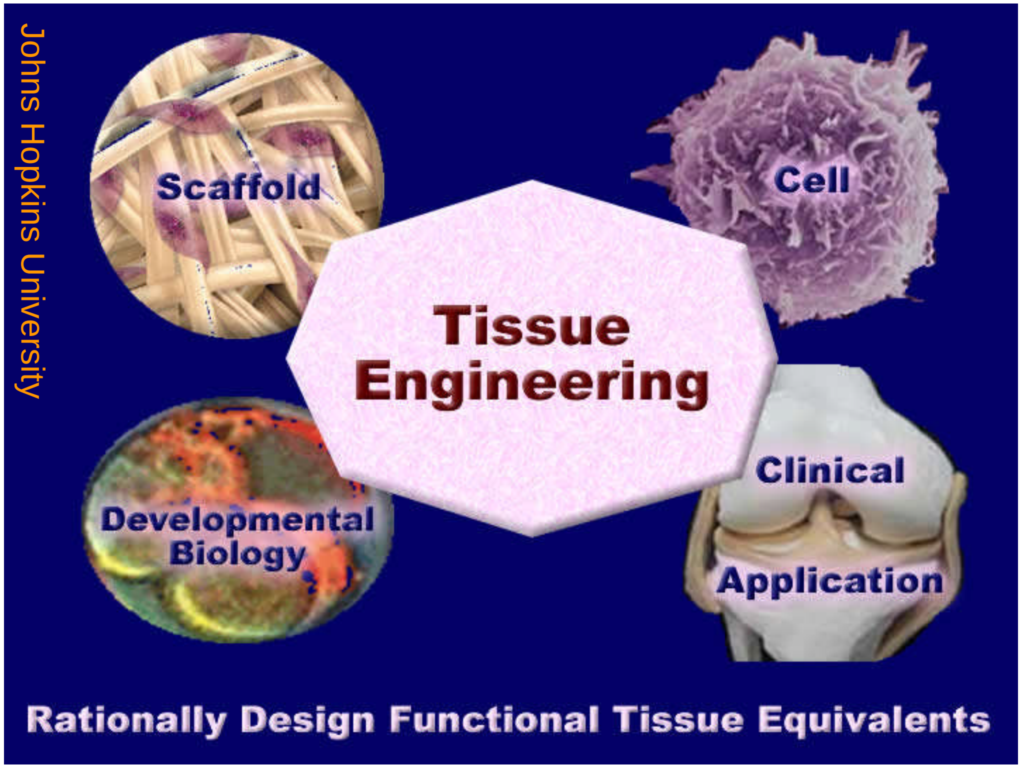

Duke University Medical Center
Orthopaedic Bioengineering Laboratory

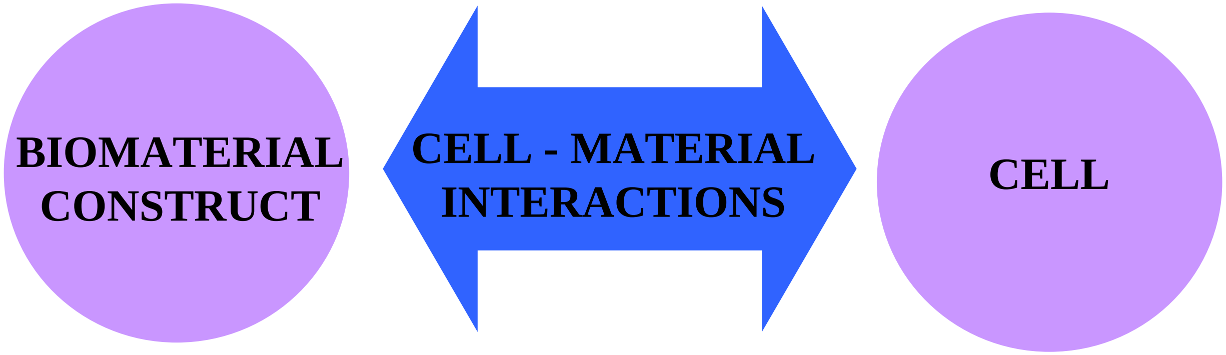
**Mouse ears**: In a tissue engineering feat, Charles Vacanti of the University of Massachusetts succeeded in growing cartilage in the shape of a human ear on the back of a mouse. The researchers used a polymer framework to grow human cartilage cells on the mouse, specially bred not to reject human cells.

Tissue engineering can perhaps be best defined as the use of a combination of cells, engineering biomaterials, suitable biochemical factors and tissue culture system to improve or replace biological functions in an effort to advance medicine.

# Tissue Engineering

NUS National University of Singapore



# Hypothesis

- Native ECM comprises of structural fibers (proteins and proteoglycans) ranging in diameter from nano- to micro-meter scale.

- Cells attach and organize well around fibers with diameters smaller than the size of the cells.

- Hypothesize that the interplay between cells and nano-scale synthetic ECM is critical to the morphological and functional development of the cells.

NUS National University of Singapore

**Cell** ⟷ **Interaction** ⟷ **Nanofiber ECM**

**Human Coronary Artery SMCs and ECs**

**Human Dermal Fibroblasts**

**Mouse Neural Cells**

**Rat Hepatocytes**

**Human Osteoblasts**

- **Morphology**
- **Gene Expression**
- **Signaling**
- **Synthesized ECM**

## Chemical Cues

**Polymer Nanofibers:**

Poly (L-lactic acid) (PLLA)

Poly ε-Caprolactone (PCL)

Co-Polymer PLLA/PCL (70:30)

Collagen

**Modified Polymer Nanofibers:**

Collagen-PCL

Collagen-PLLA/PCL

Galactosylated-PCL

$CaCO_3$-PCL

## Physical Cues

**Fiber Diameter (nano vs micro vs solvent cast film**

**Random Nanofibers**

**Aligned Nanofibers**

**Patterned Nanofibers**

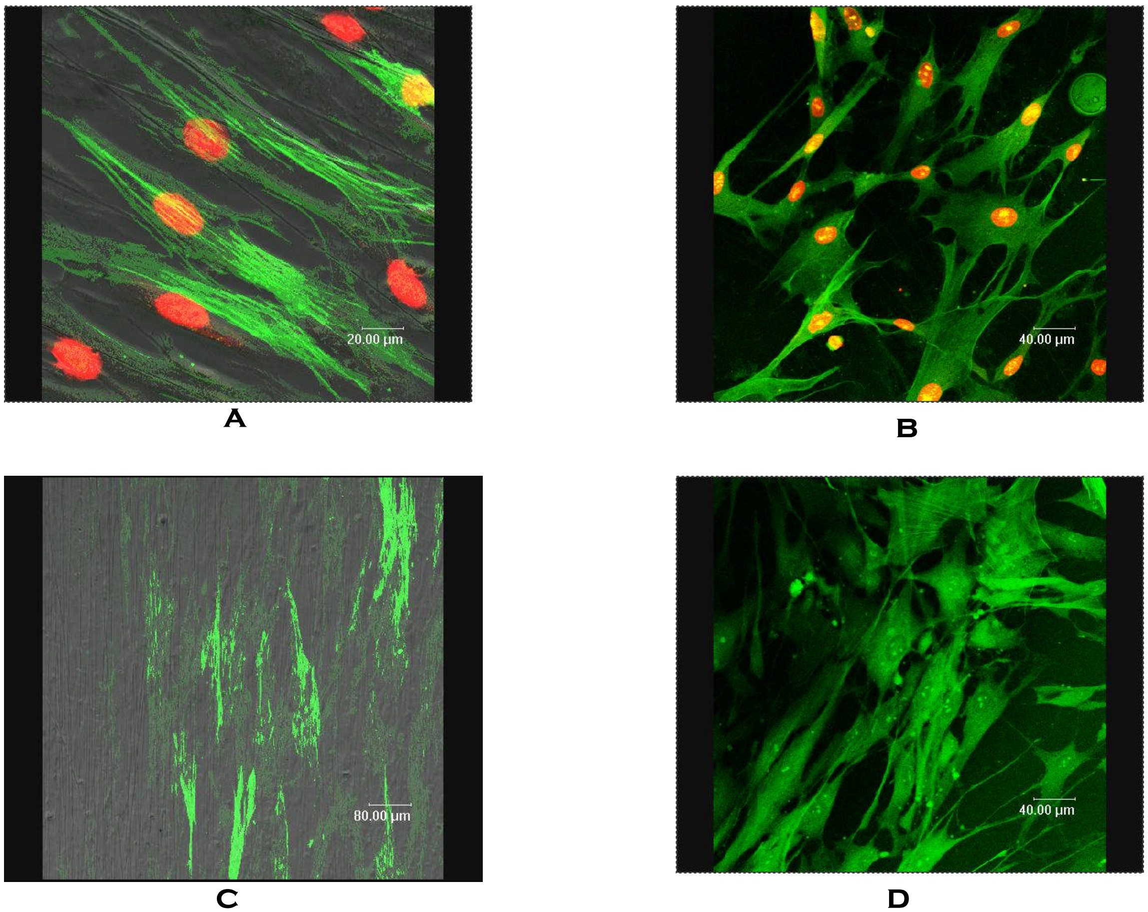
**3-Dimensional Nanofibrous Scaffolds**

*AISB4 & FNB2004, 16-18 Nov 2004*

***ICNT, Nov 7-11, 2004***

# Cell Morphology – SMCs

## Successful Culturing of SMCs on Aligned nanofibers




The material used was
co-polymer PLLA/CL (70:30):

a). α-actin filaments
on aligned nanofibrous scaffold.

b). α-actin filaments on TCPS.

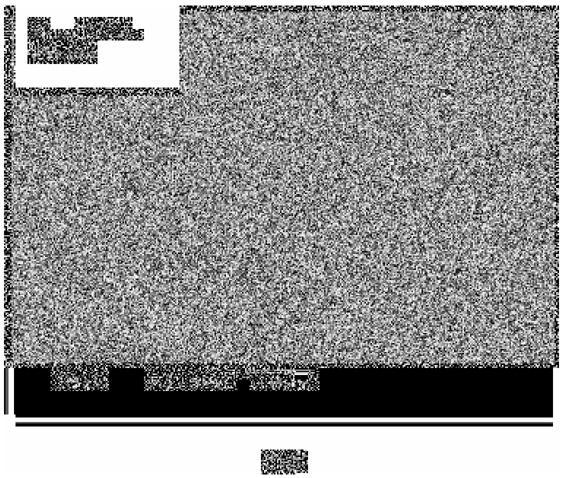
c). Myosin filaments
on aligned nanofibrous scaffold.

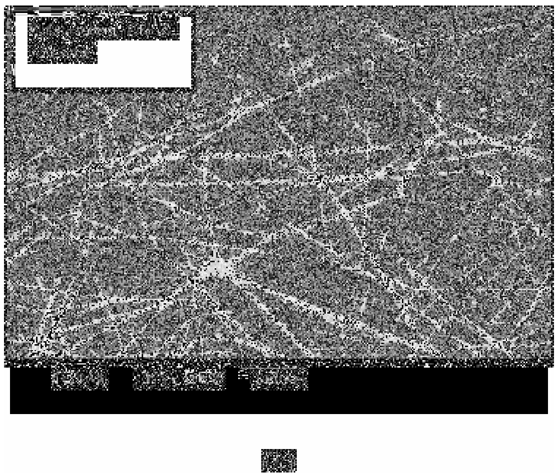
d). Myosin filaments on TCPS.

*Xu C.Y., Inai R., Kotaki M. and Ramakrishna S., Aligned Biodegradable Nanofibrous Structure: A Potential Scaffold for Blood Vessel Engineering, Biomaterials, 25, 5, (2004) 877-886.*

# Cell Morphology – SMCs

## Effects of Fiber Dimensions





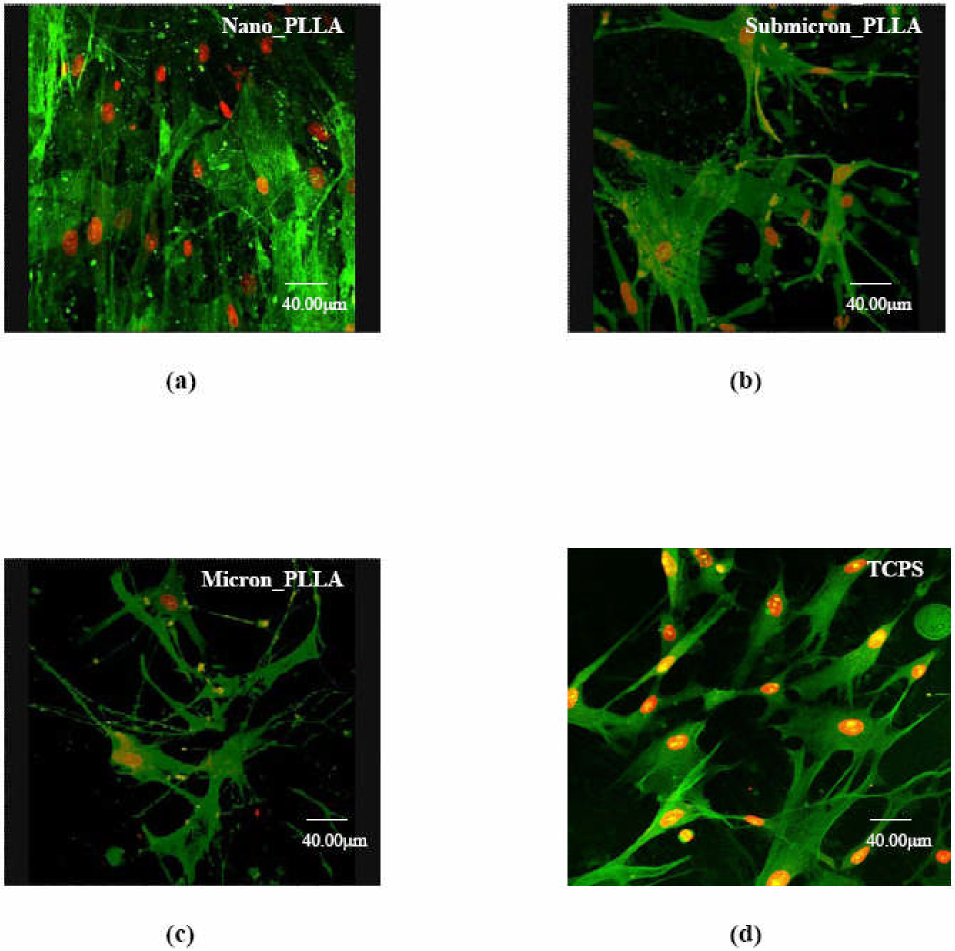
The material used was PLLA:

a). Nano Dimension.

b). Sub-Micron Dimension.

c). Micron Dimension.

# Cell Morphology – SMCs
## Effects of Fiber Dimensions




The material used was PLLA:

a). Nano Dimension.

b). Sub-Micron Dimension.

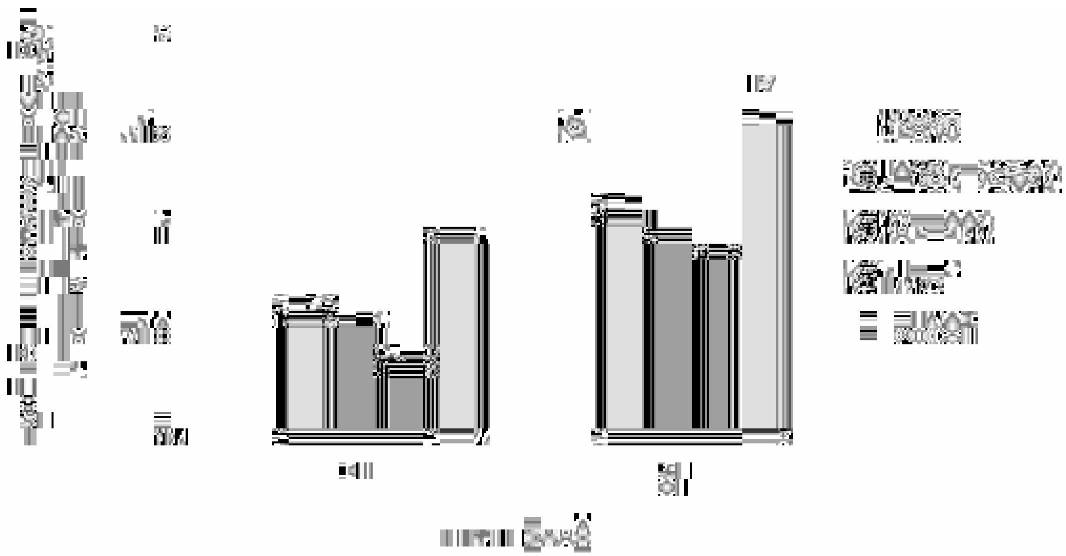
c). Micron Dimension.

d). Tissue Culture Plate.

**Nano dimension fibers provide the best support for SMCs growth.**

# Cell Morphology – SMCs

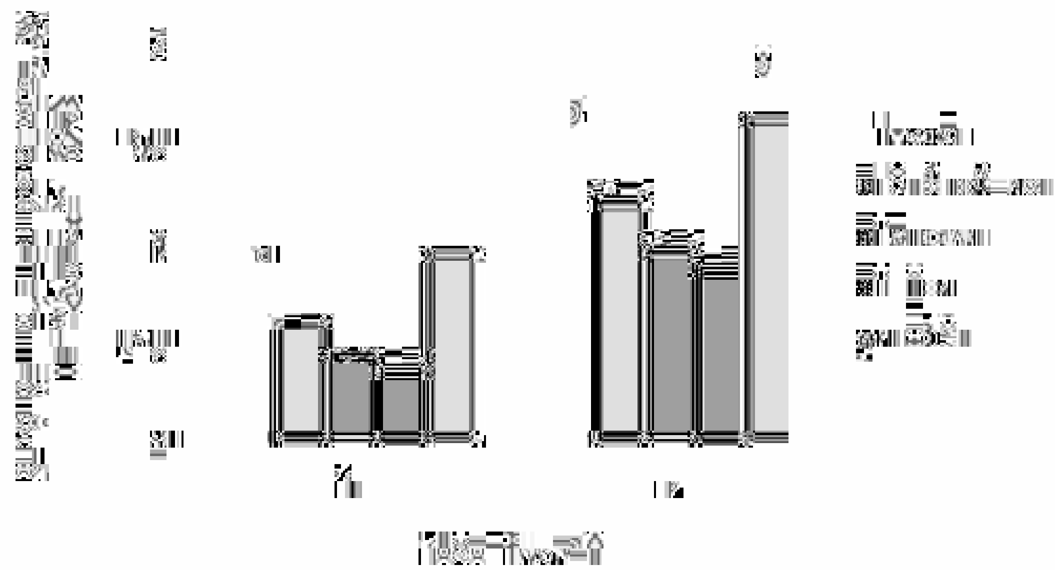
## Effects of Fiber Dimensions on Cell Adhesion



Nano-dimension fibers provide better SMCs adherence than sub-micron and micron dimension PLLA fibers.

# Cell Morphology – SMCs

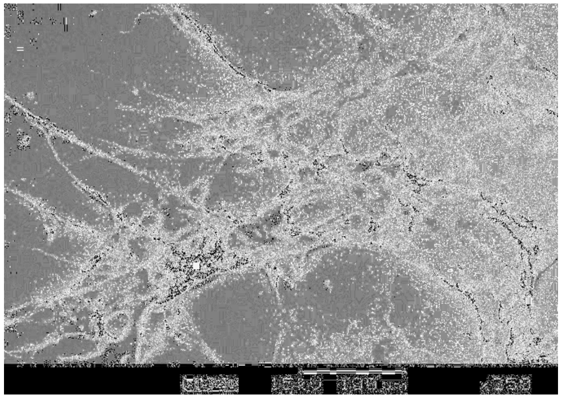
## Effects of Fiber Dimensions on Cell Proliferation



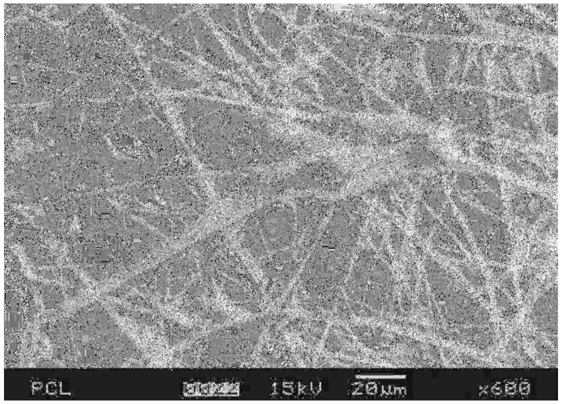
Nano-dimension fibers support better SMCs proliferation than sub-micron and micron dimension PLLA fibers.

# Cell Morphology – SMCs

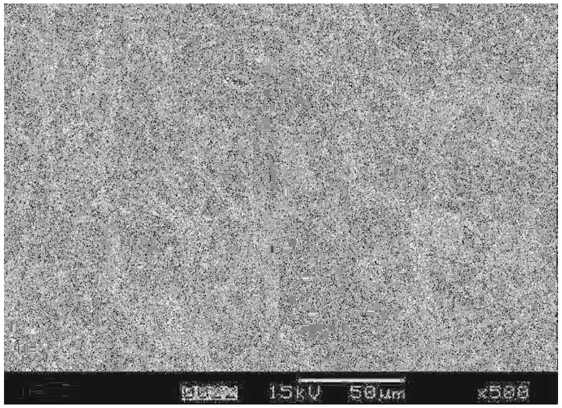
## SMCs Cultured on Collagen Nanofibers



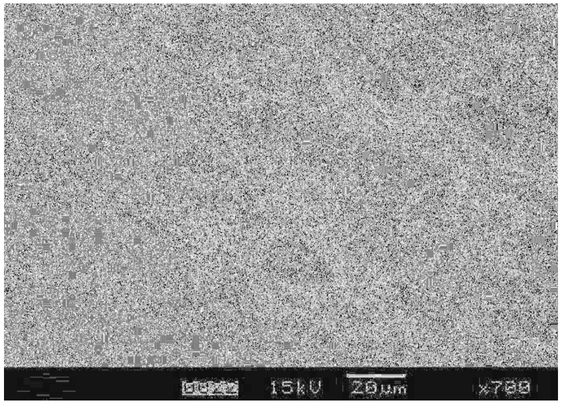
Control




PCL




PCL + Collagen




Collagen

SEM micrographs of SMCs cultured on:

a). Control tissue culture plate.

b). PCL nanofibers.

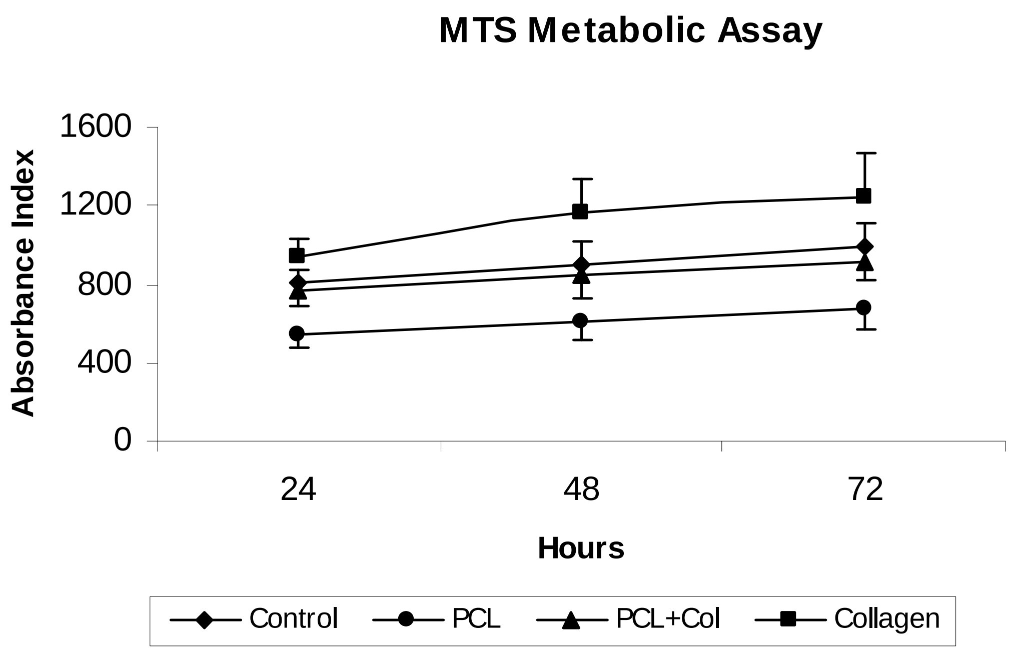
c). Collagen coated PCL nanofibers.

d). Collagen nanofibers.

Collagen nanofibers provide the best support for SMCs proliferation.

# Cell Morphology – SMCs

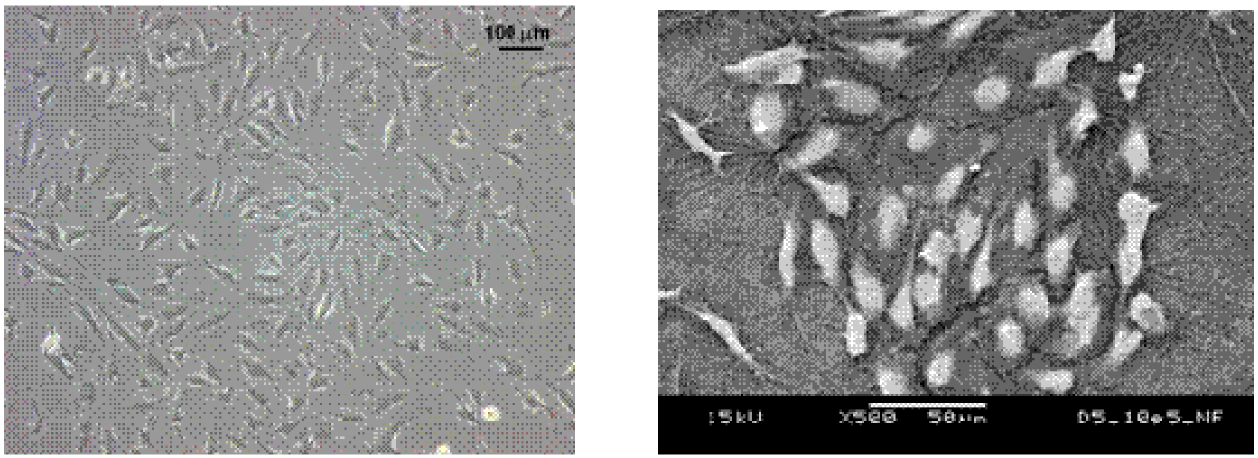
## SMCs Cultured on Collagen Nanofibers - Cell Proliferation




By MTS assay, collagen nanofibers provide the best support for SMCs proliferation.

## Cell Morphology – ECs

### ECs Cultured on PLLA/PCL Co-Polymer Nanofibers

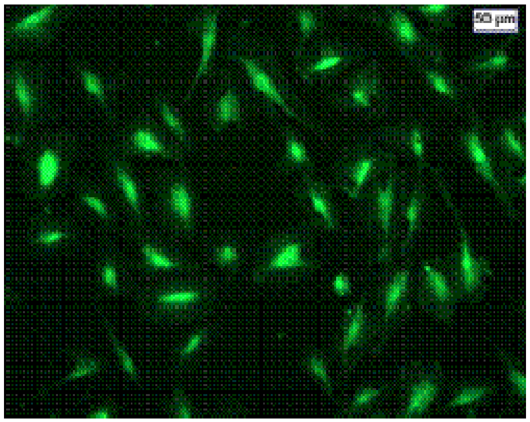



A B

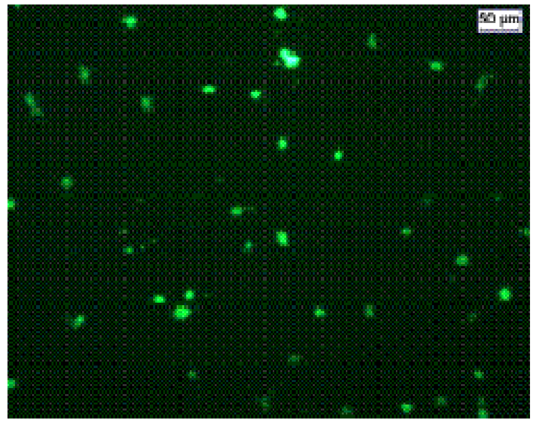
**We observed that ECs do not grow very well on unmodified nanofibers (b) and exhibit a more rounded morphology when compared to ECs cultured on tissue culture plates (a). Endothelialization of material surface is essential to prevent thrombosis and enhance graft survival.**

# Cell Morphology – ECs

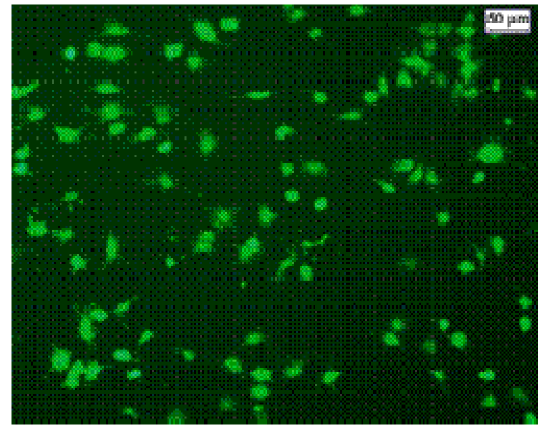
## ECs Cultured on Collagen-Modified PLLA/PCL Co-Polymer Nanofibers



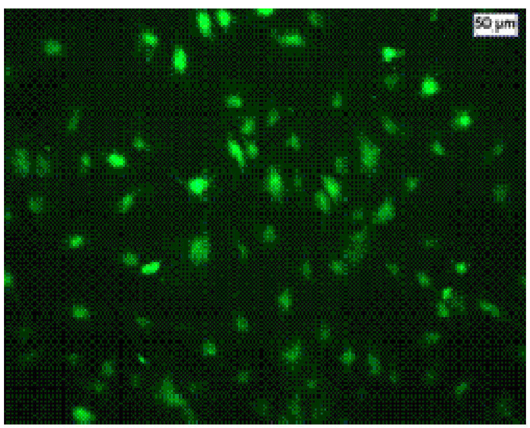
Tissue Culture plate



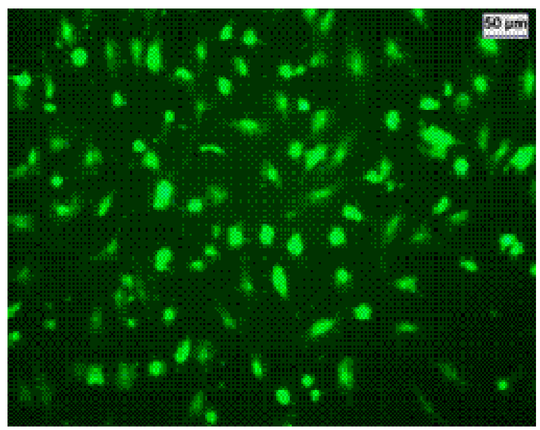
Unmodified Nanofiber



Plasma Treated Nanofiber



Collagen Coated Nanofiber



Collagen Blended Nanofiber

CMFDA STAINED CELLS DAY 1 POST SEEDING

**ECs cultured on collagen-modified nanofibers exhibit very similar morphological resemblance to that on TCPS**

*He W., Yong T., Teo W.E., Ma Z. and Ramakrishna S., Fabrication and endothelialization of collagen-blended biodegradable polymer nanofibers: potential vascular graft for the blood vessel tissue engineering, Tissue Engineering, (submitted).*

# Cell Morphology – Mouse Neural Cell Line c17.2

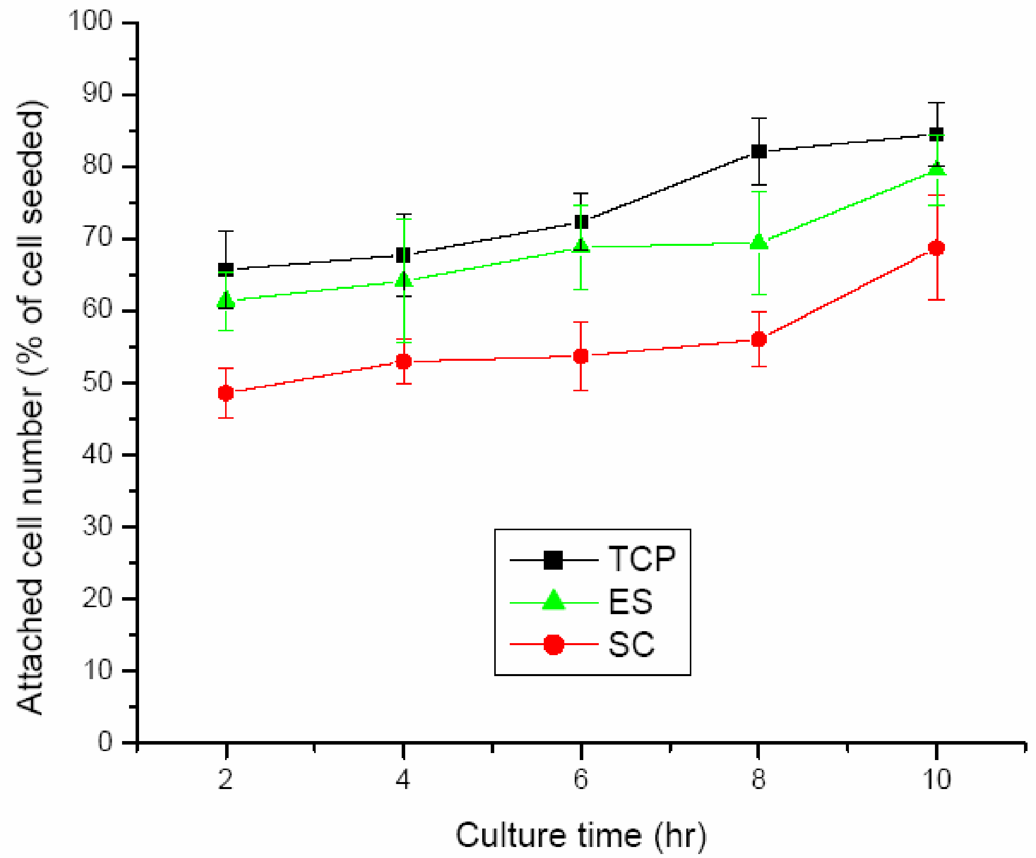
## Successfully Cultured Neural Cells on Random PLLA Nanofibers

**THE NEURITE EXTENDED MORE THAN TWICE THE LENGTH OF THE CELL BODY AFTER 3 DAYS OF CULTURE.**

*Yang F., Murugan R. Wang S. and Ramakrishna S., Electrospinning of nano/micro scale poly (L-lactic acid) aligned fibers and their potential in neural tissue engineering, Biomaterials, (in press).*

# Cell Morphology – Mouse Neural Cell Line c17.2

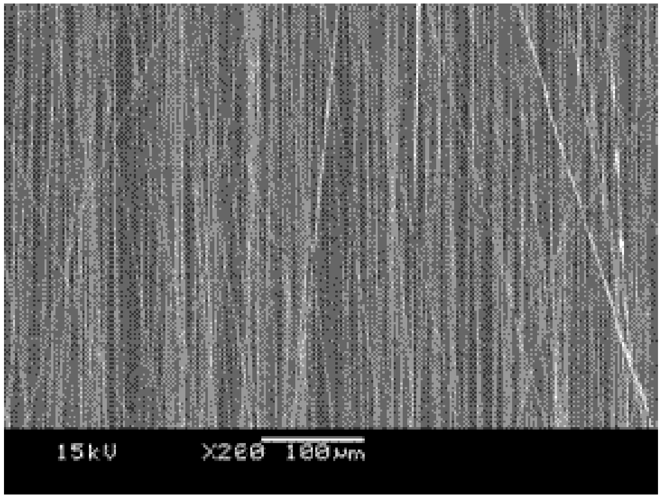
## Neural Cells Cultured on PLLA Nanofibers - Cell Adhesion



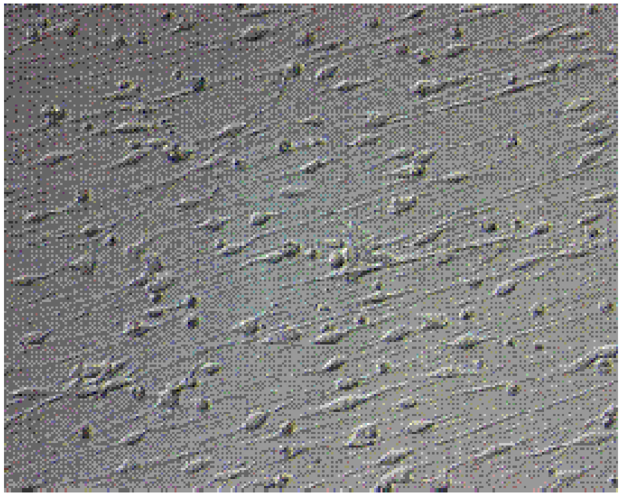

Neural stem cells adhered better to nanofibers (ES) than solvent cast film (SC). TCP = tissue culture plate.

# Cell Morphology – Mouse Neural Cell Line c17.2

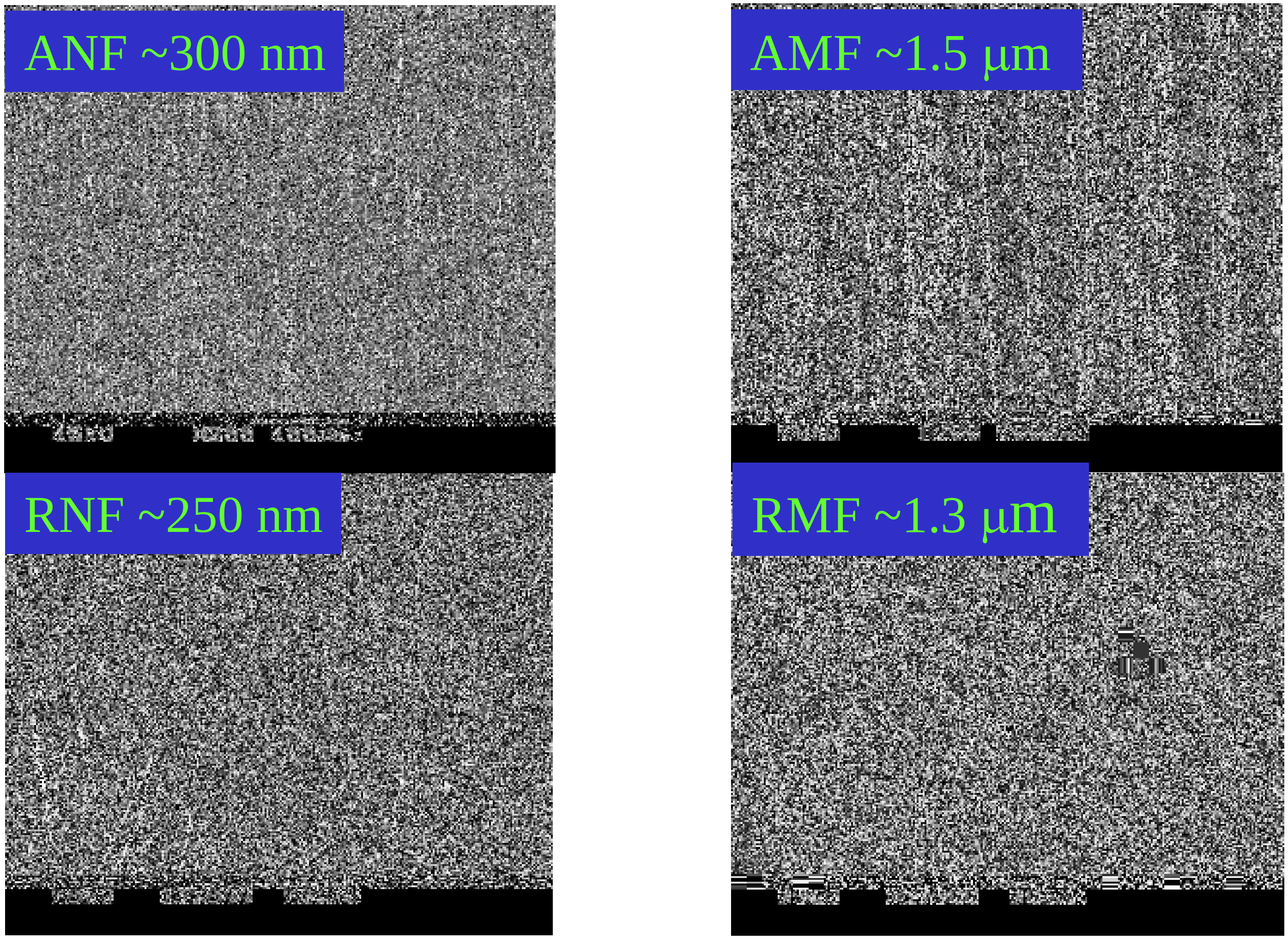
## Successfully Cultured Neural Cells on Aligned PLLA Nanofibers




SEM photographs of PLLA aligned nanofibers

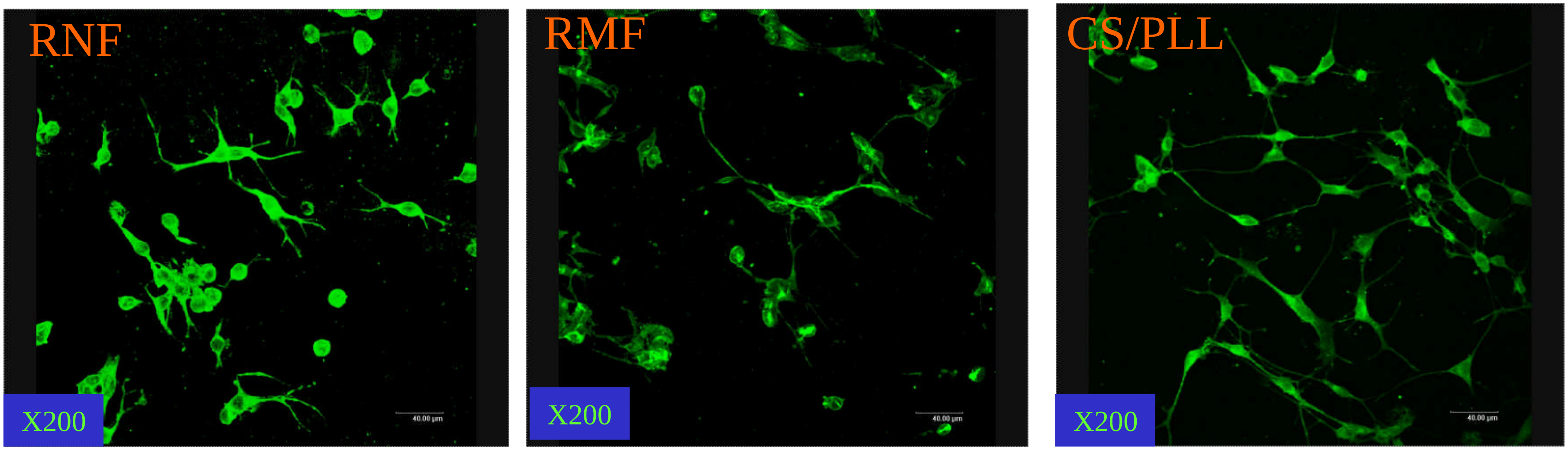


2 days after seeding with neural stem cells



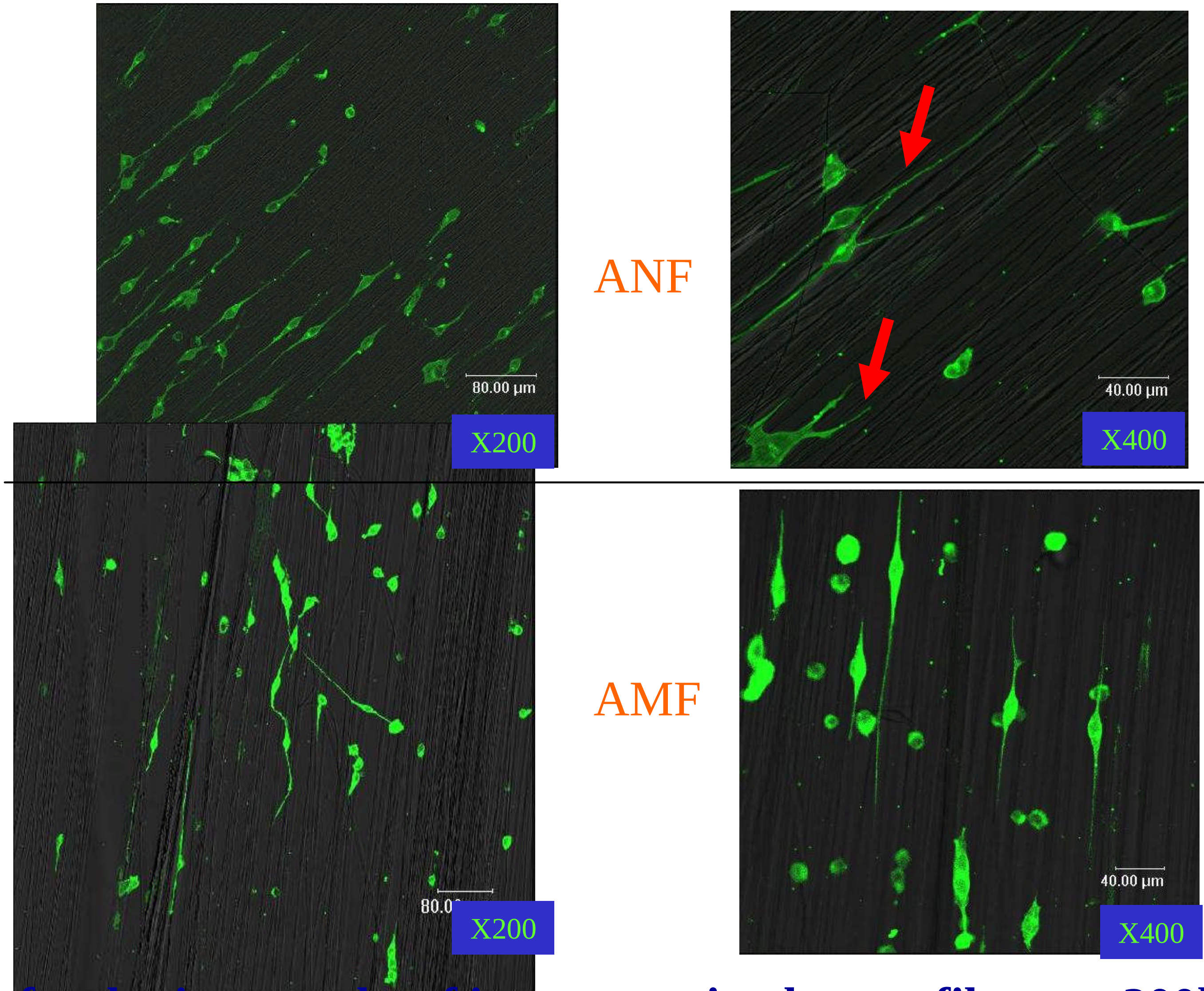

**SEM micrographs of electrospun PLLA fibers**

**ANF:** Aligned nanofibers **AMF:** Aligned micro fibers
**RNF:** Random nanofibers **RMF:** Random micro fibers




**Confocal micrographs of immunostained neurofilament 200kD in NSCs after 2 days of culture on random PLLA fibers and the glass coverslip coated with poly-L-lysine (CS/PLL) used as control**

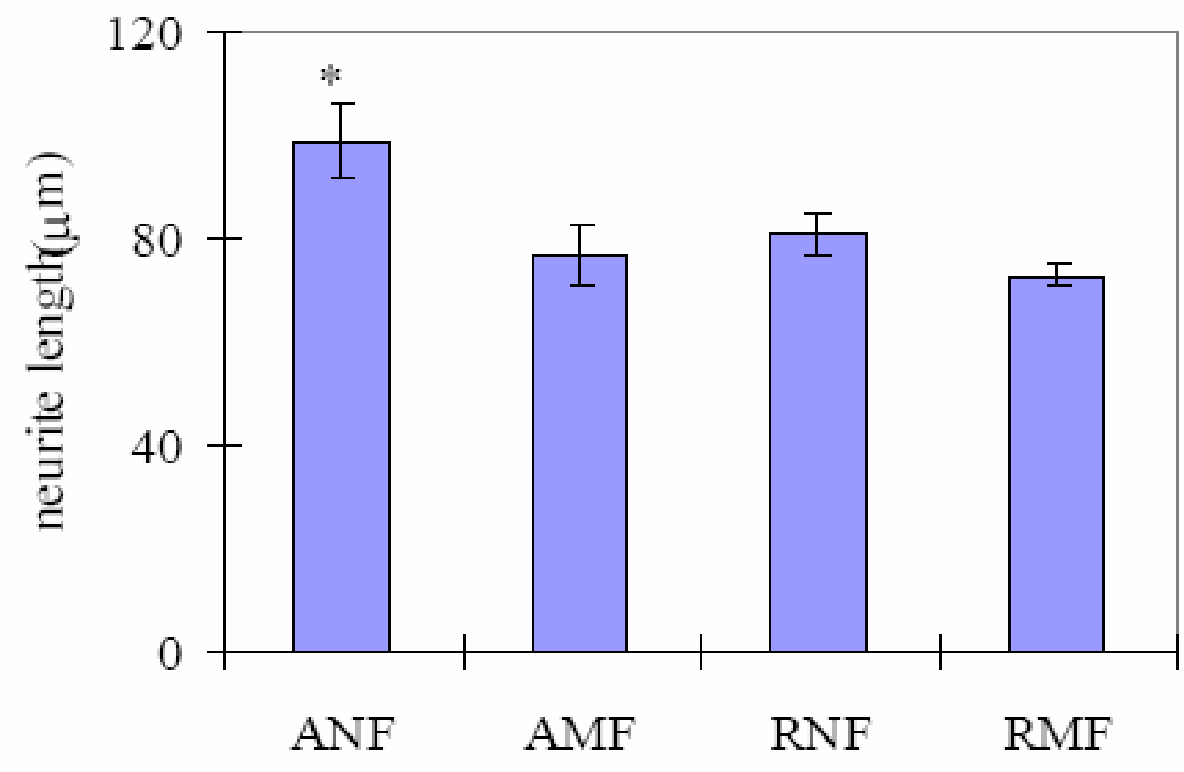
- Neurites are ***randomly*** orientated.
- NSCs present ***multiple*** and ***branched*** processes.



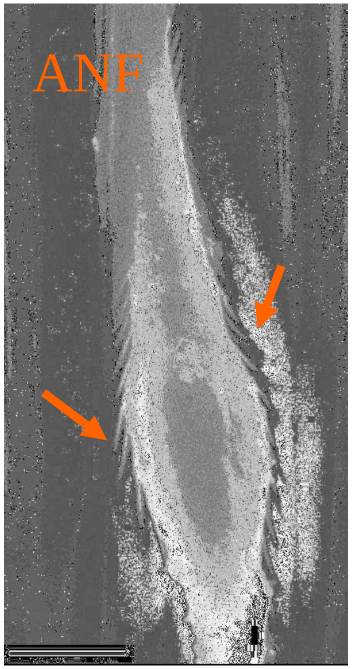

**Confocal micrographs of immunostained neurofilament 200kD in NSCs after 2 days of culture on aligned PLLA fibers**

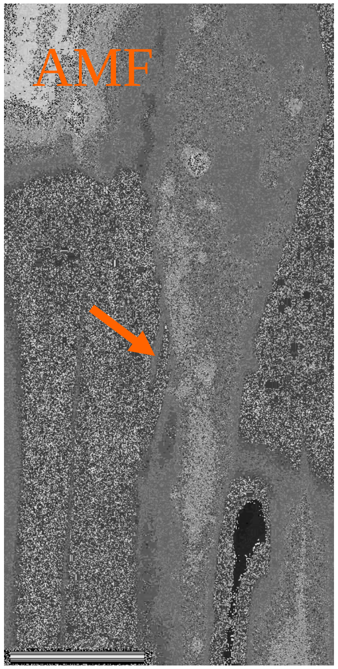
# Cell Morphology – Mouse Neural Cell Line c17.2

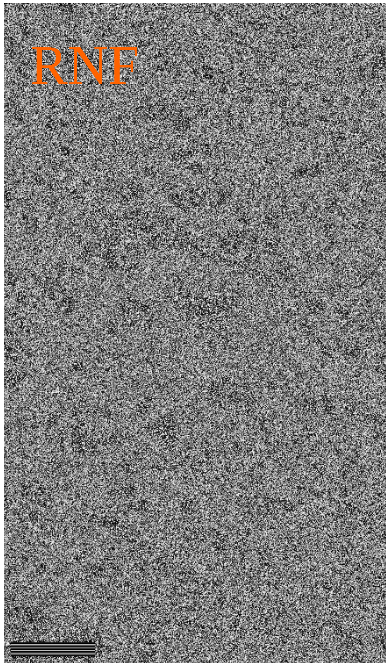
## Neural Cells Cultured on PLLA Nanofibers – Neurite Length

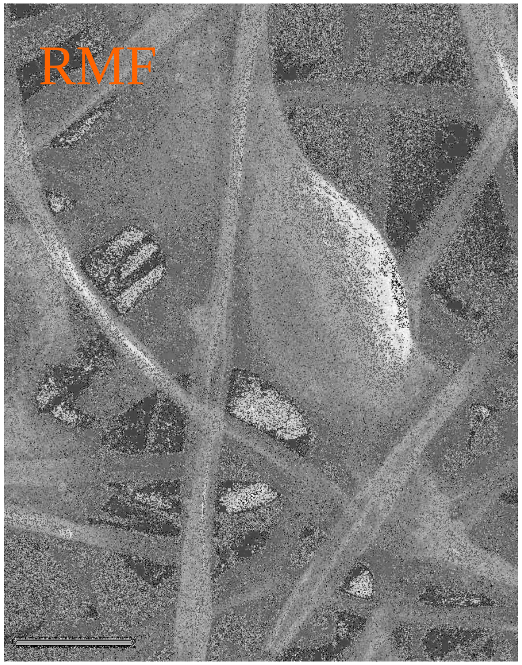


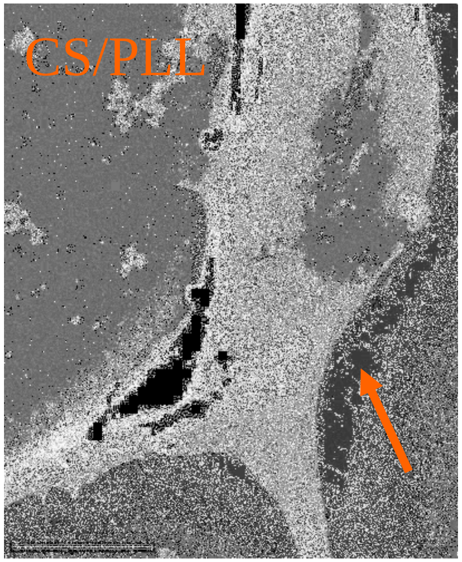

On aligned nanofibers (ANF), neural stem cells established the greatest neurite length. AMF = aligned microfibers, RNF = random nanofiber, RMF = random microfibers.



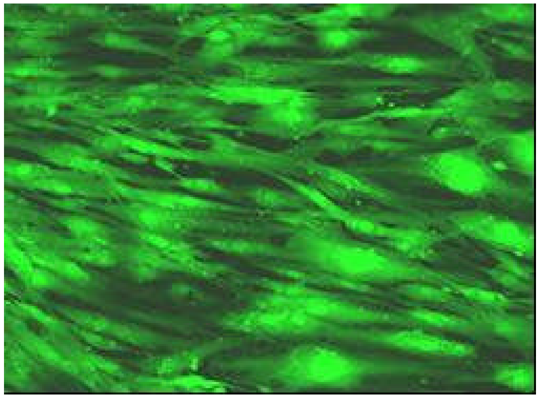



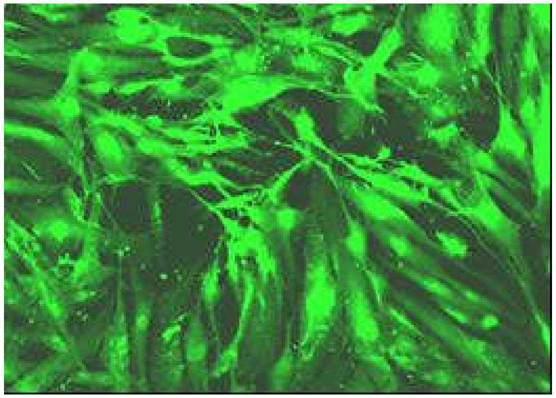

**SEM micrographs of NSCs seeded on different substrates for 2 days showing the cell-matrix adhesion between the NSCs and the PLLA fibers.**
**Bar = 5 μm.**

# Cell Morphology – Human Dermal Fibroblasts

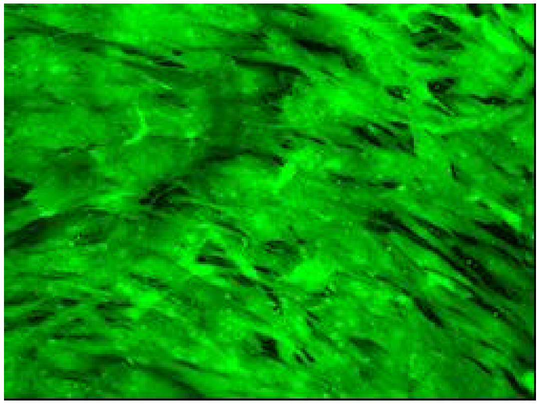
## HDFs Cultured on Collagen Nanofibers



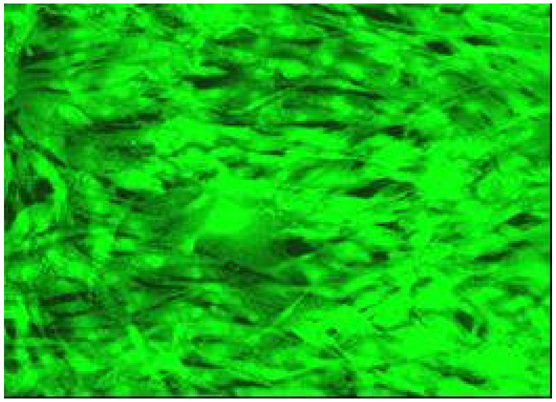
Tissue Culture Plate



PCL



Collagen-Coated PCL

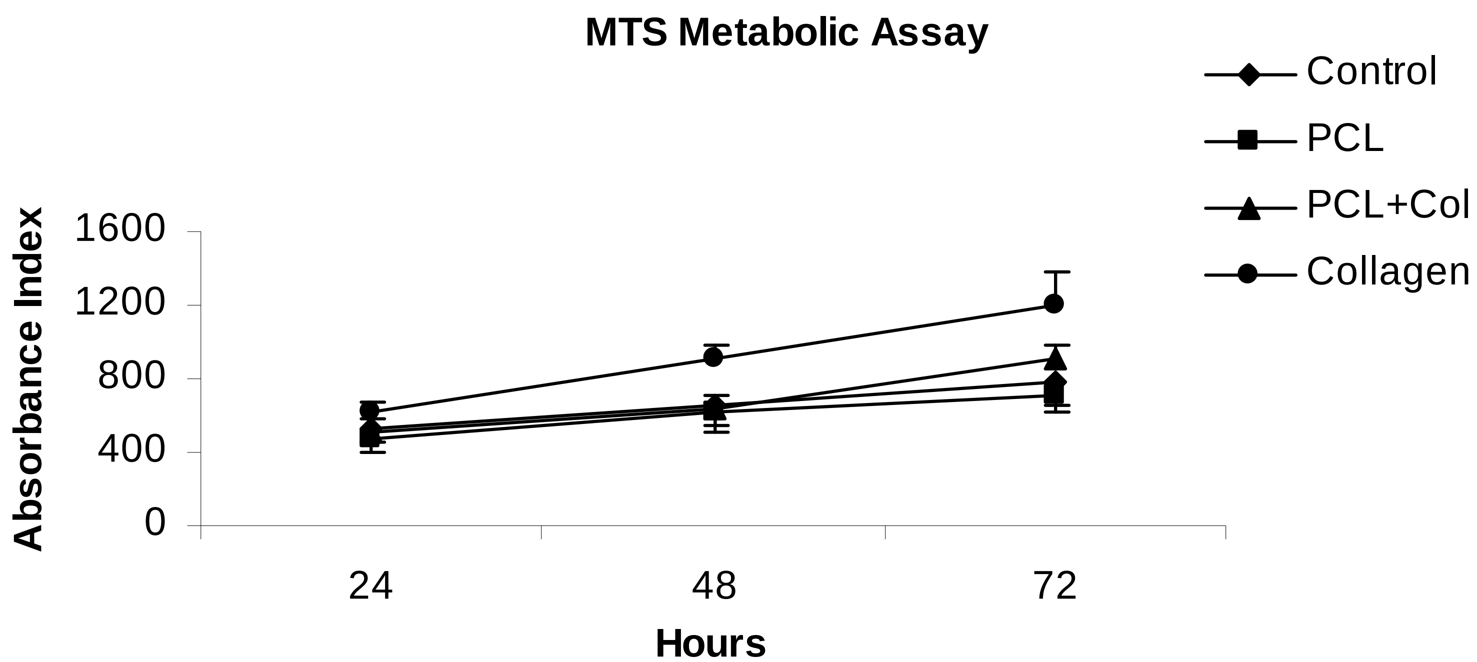


Collagen

Collagen nanofibers provide the best support for HDFs proliferation.

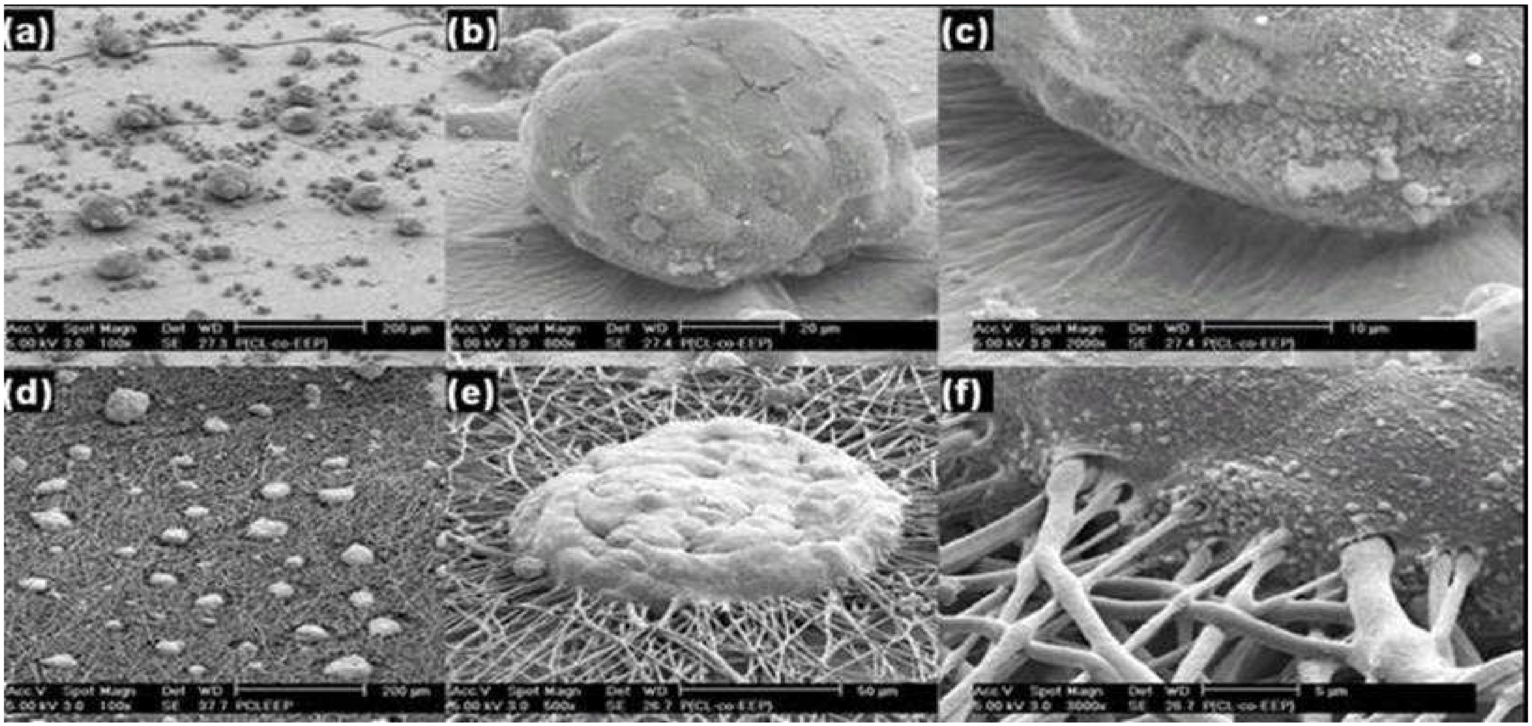
# Cell Morphology – Human Dermal Fibroblasts

## HDFs Cultured on Collagen Nanofibers - Cell Proliferation




By MTS assay, collagen nanofibers provide the best support for HDFs proliferation.

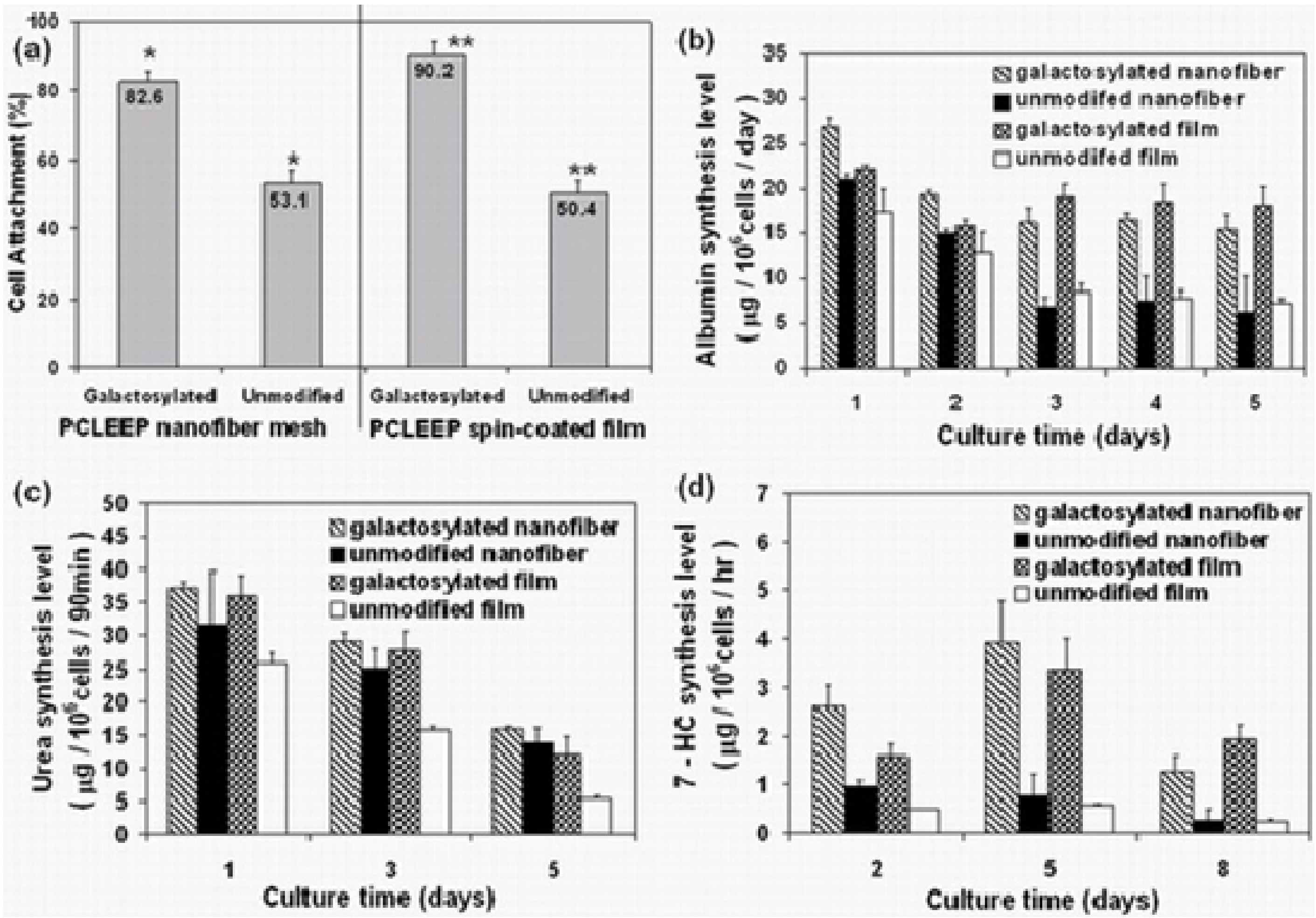
# Cell Morphology – Hepatocytes



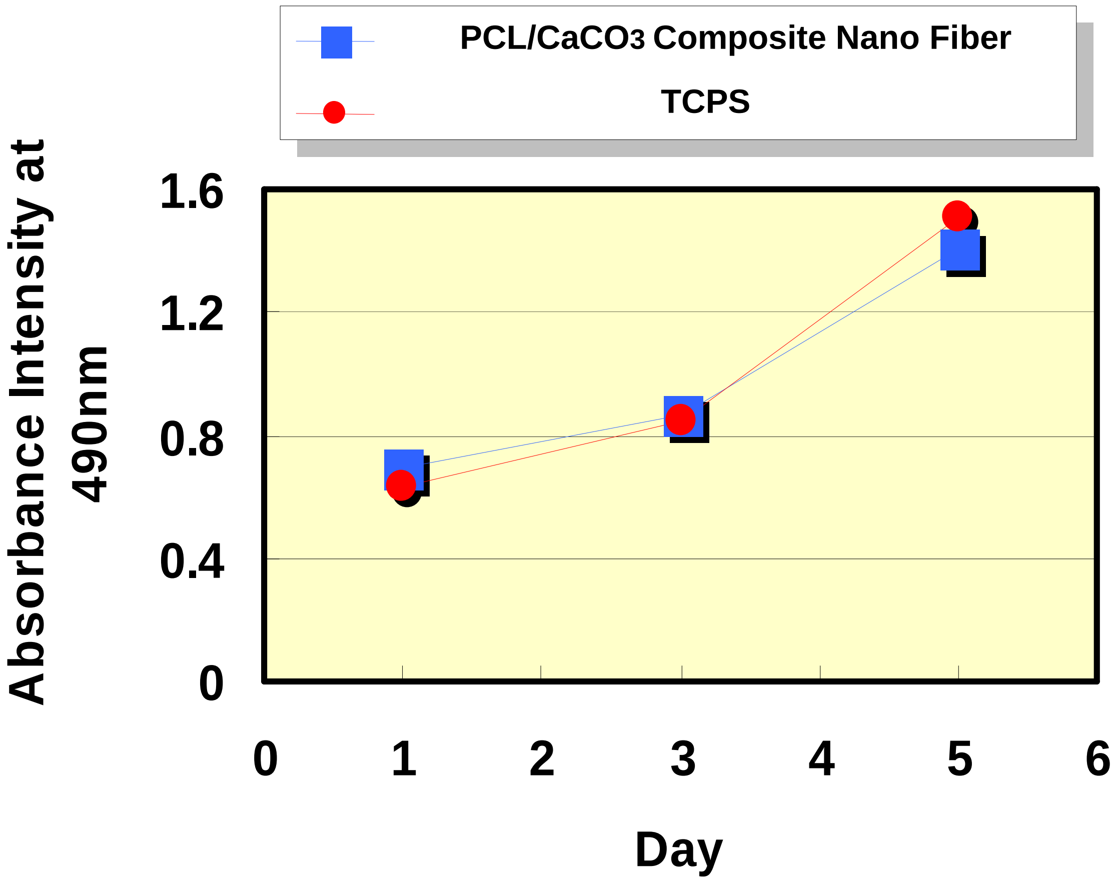

**SEM images of hepatocytes after 8 days of culture: (a, b, c) Hepatocytes cultured on galactosylated PCLEEP film formed rounded spheroids that did not integrate with the scaffold; (d, e, f) In contrast, hepatocytes cultured on galactosylated PCLEEP nanofiber mesh showed that the aggregates engulfed the functional nanofibers**

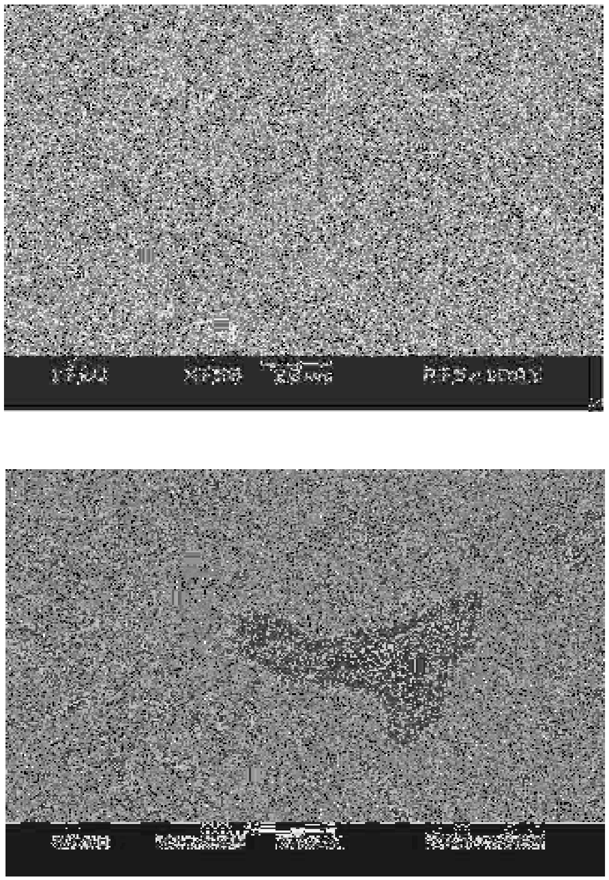
*Chua K.N., Lim W.S., Zhang P.C., Lu H.F., Wen J., Ramakrishna S., Leong K.W. and Mao H.Q., Stable immobilization of rat hepatocyte spheroids on galactosylated nanofiber scaffold, Biomaterials, (in press).*

# Cell Morphology – Hepatocyte Cell Attachment




**(a)** Hepatocyte attachment on galactosylated and unmodified nanofiber meshes and spin-coated films 3 hours after seeding. Data are means ± SD, n = 6. *p < 0.01; **p < 0.01. **(b)** Albumin secretion levels, **(c)** urea synthesis function levels, and **(d)** 3-MC induced P450 function levels of hepatocytes at various time points normalized against the total number of attached cells **(a)** when cultured on different substrates. Data are means ± SD, n = 2.



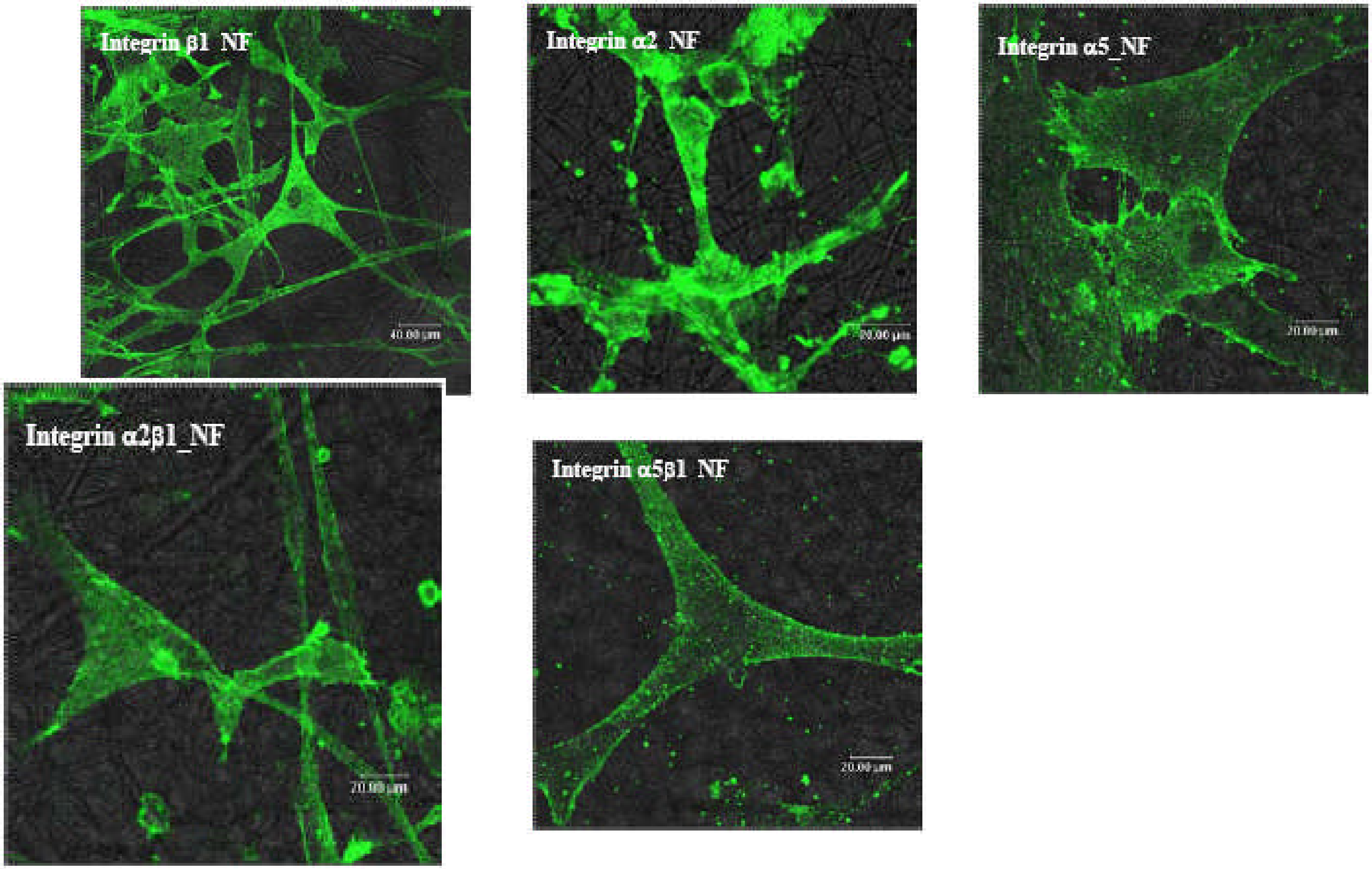
Day 1

*Human osteoblasts cultured on PCL/$CaCO_3$ composite nanofiber scaffolds exhibited excellent cell attachment.*

## Gene Expression

- Cell Adhesion Gene Expression Analysis
- By Immunofluorescence and Flow Cytometry
  - Integrins – $\alpha$ and $\beta$
- By RT-PCR
  - Integrins – $\alpha$ and $\beta$
  - PECAM-1
  - ICAM-1
  - VCAM-1
  - von Willebrand Factor (vWF)
  - $\beta$-Actin (control)

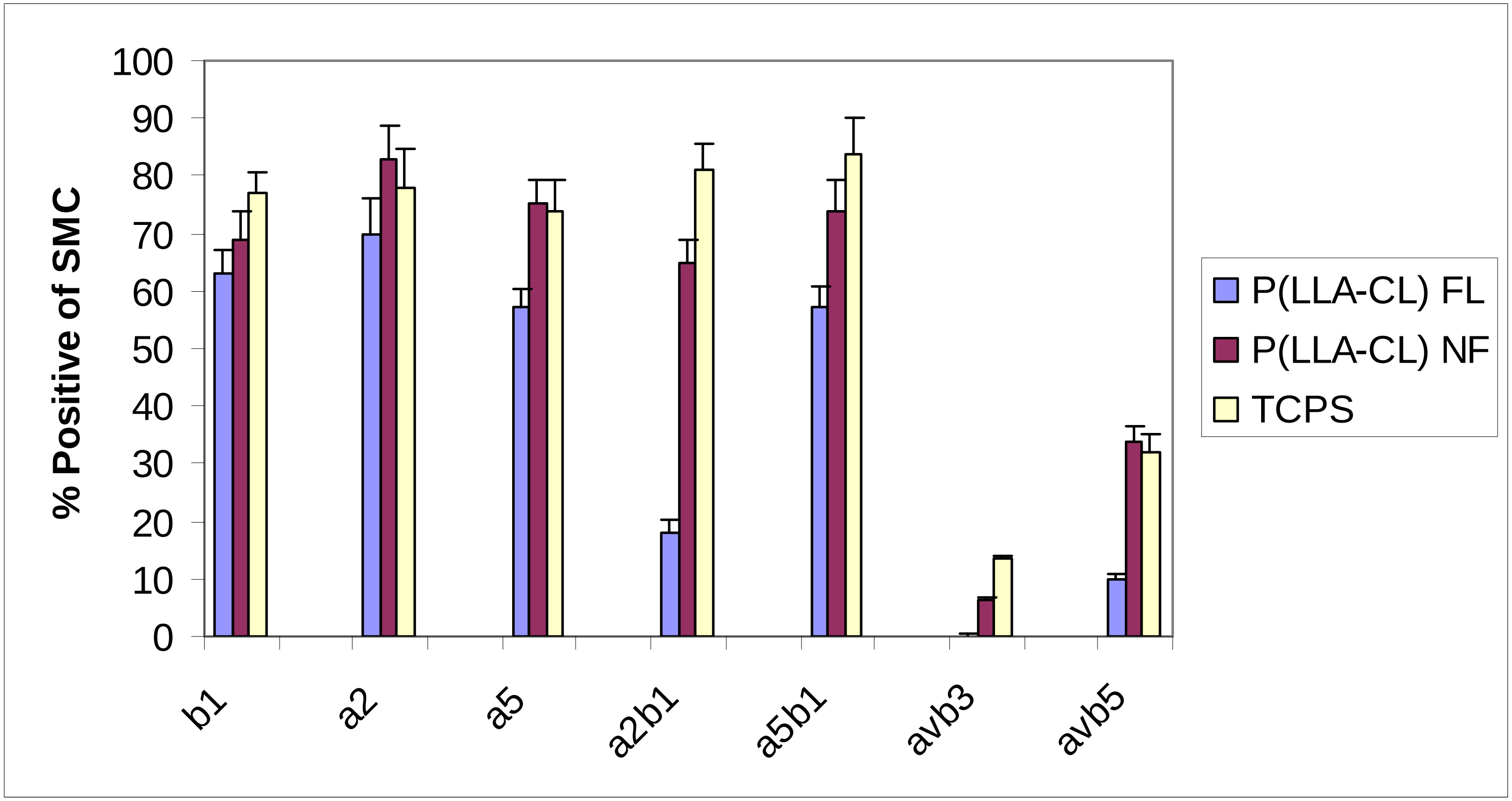
# Gene Expression – SMCs: Immunofluorescence




**LSCM micrographs of immunofluorescent staining using antibodies against β1, α2, α5, α2β1 and α5β1 integrins on SMCs cultured 24 hours on nanofibrous P(LLA-CL) substrate (NF)**

# Gene Expression - SMCs

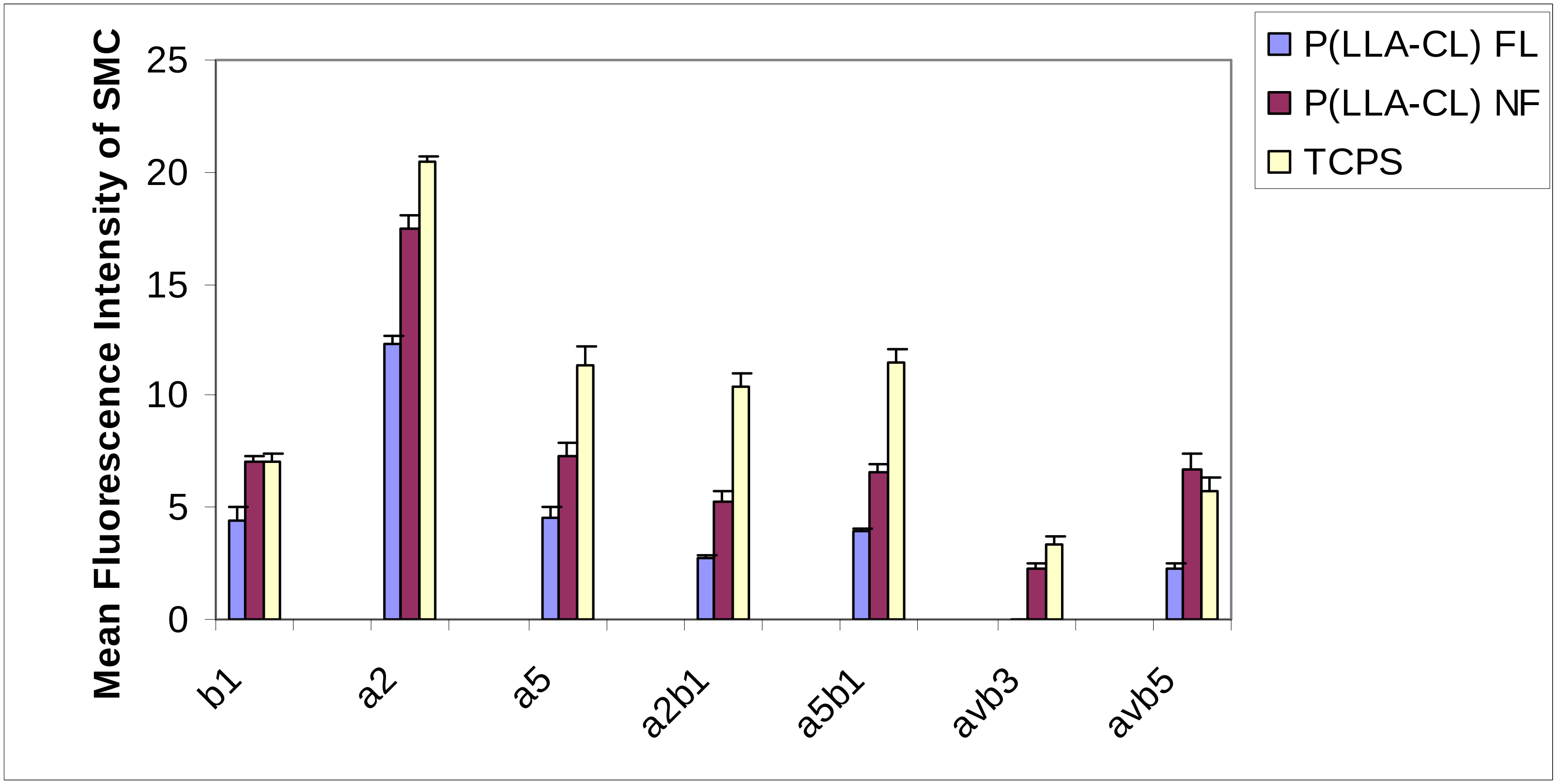
*Flow Cytometry: Data Analysis*




**The percentage of positive SMCs cultured on different substrates which expressed different adhesion proteins.**

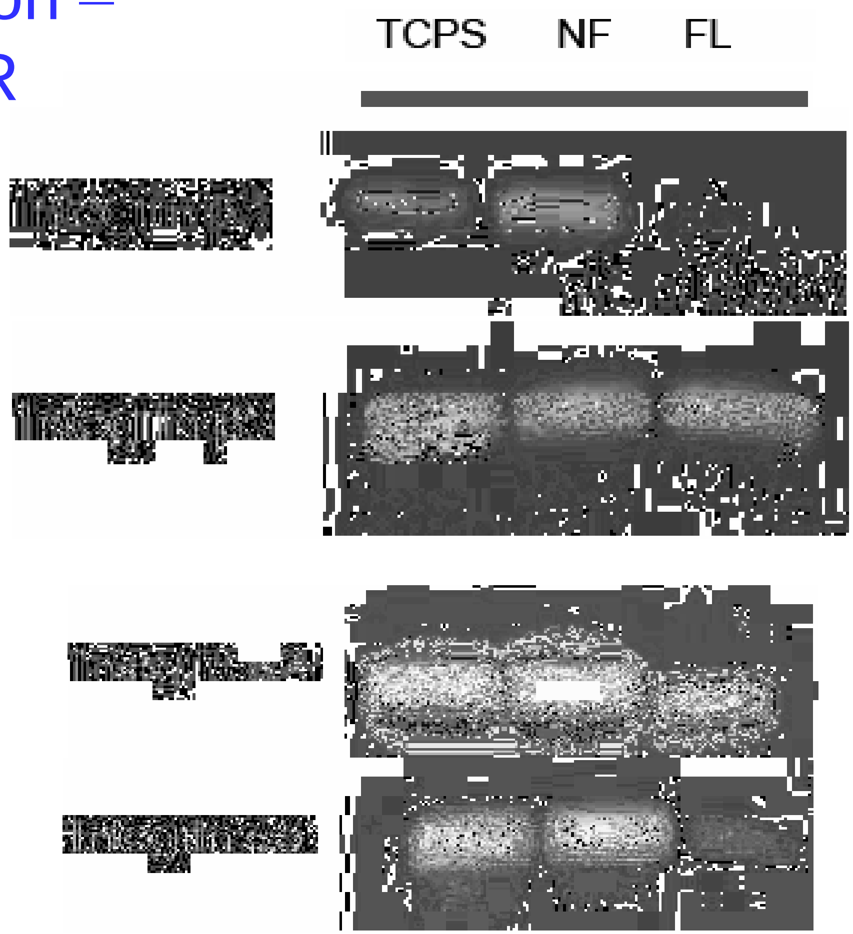
# Gene Expression - SMCs

## *Flow Cytometry: Data Analysis*




**The intensity of adhesion proteins expressed by SMCs cultured on electrospun nanofibrous PLLA:PCL scaffold, solvent cast PLLA:PCL film and TCPS substrate**

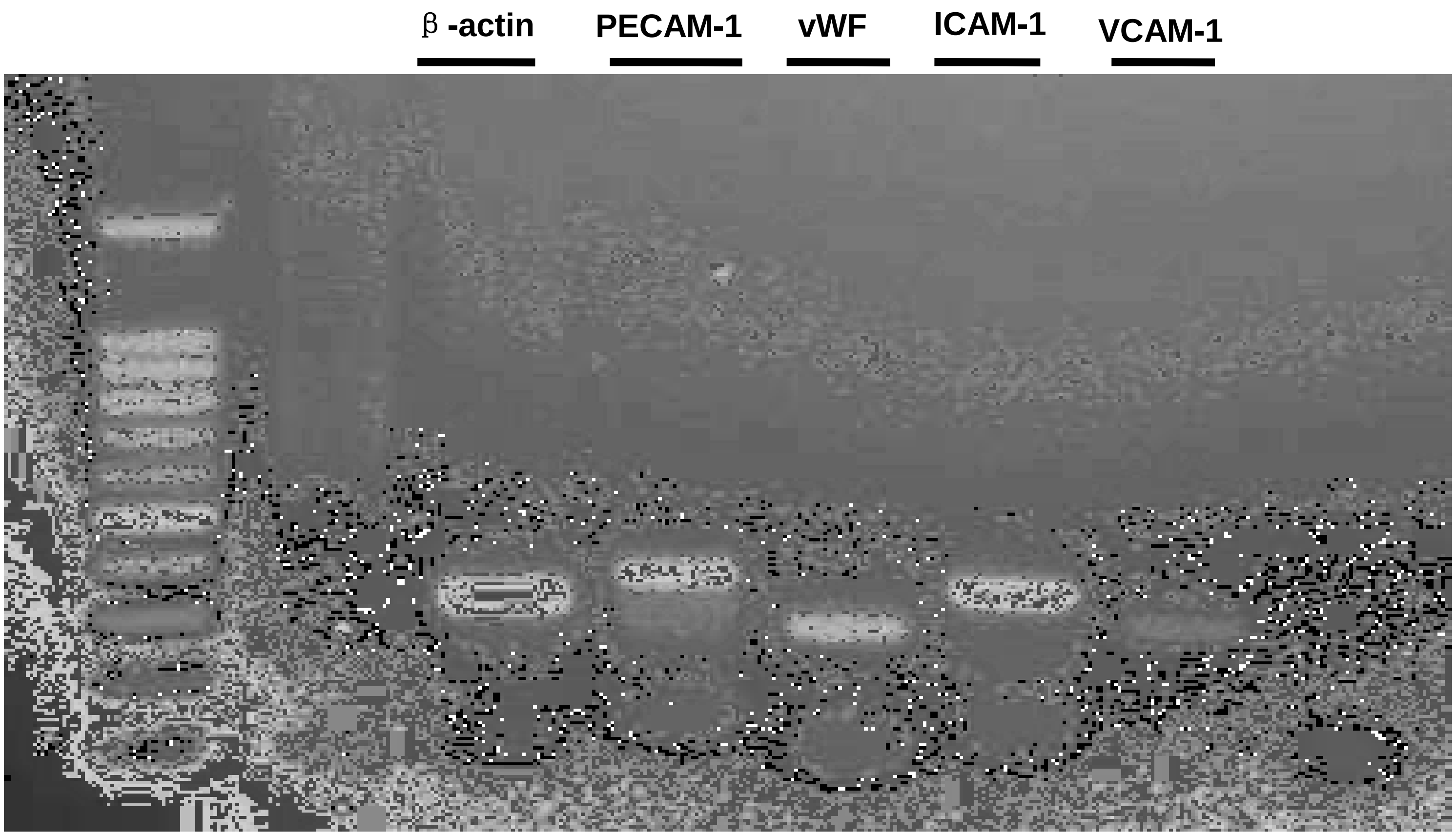
# Gene Expression – SMCs: RT-PCR




**RT-PCR analysis of integrin subunits β1, β3, β5, α2 and α5 mRNA expression in SMCs cultured on tissue culture polystyrene substrate (TCPS), nanofibrous P(LLA-CL) substrate (NF) and conventional P(LLA-CL) film substrate (FL) 24 hours post seeding.**

# Gene Expression – ECs: RT-PCR

*ECs Cultured on Collagen:Co-Polymer Blended Scaffolds*

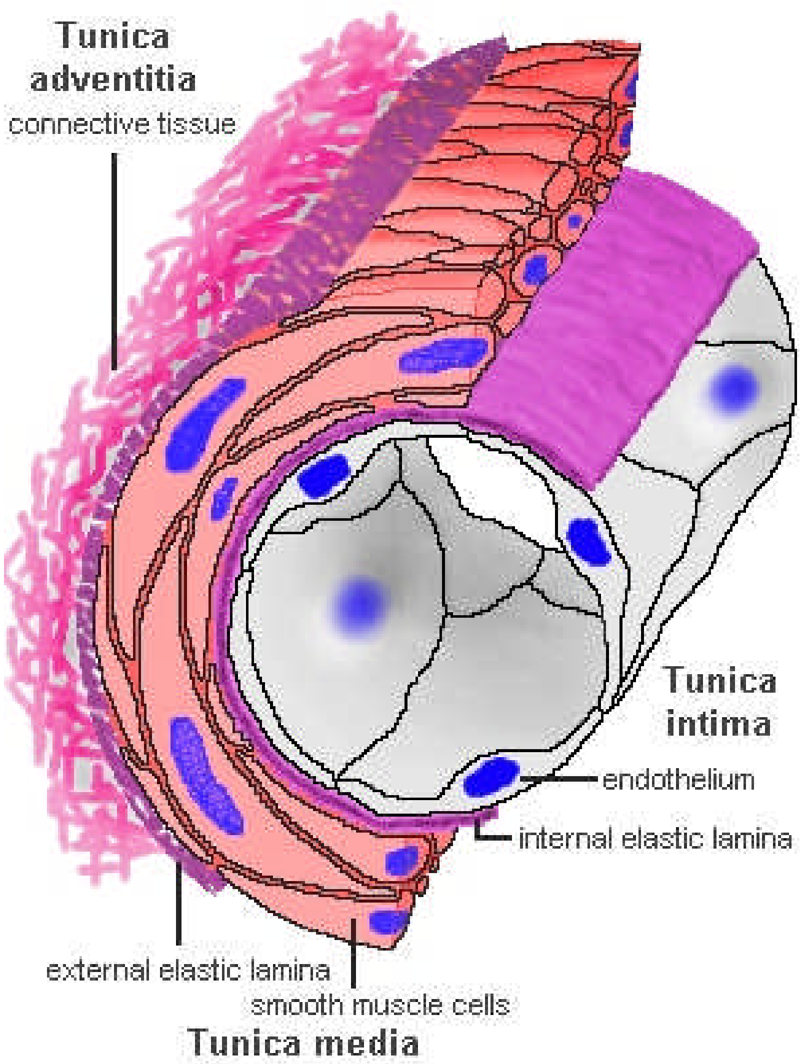



**Preliminary data suggests collagen:co-polymer blended scaffolds provide good support for ECs growth.**

# Tissue Engineering - Conclusions

- SMCs proliferate well on unmodified synthetic nanofibers and orientate along the alignment of the nanofibers.
- ECs require modified synthetic nanofibers for growth.
- Neural cells grow well on unmodified synthetic nanofibers and aligned nanofibers promote neurites extension.
- Collagen nanofibers promote excellent growth of HDFs.
- Galactosylated nanofibers encourage integration of hepatocyte aggregates into the mesh.
- Calcium carbonate-nanofiber composite nanofiber membrane promotes osteoblasts growth.
- Adhesion molecules and ECM proteins are well expressed by SMCs and ECs cultured on nanofibers.

# Blood Vessel

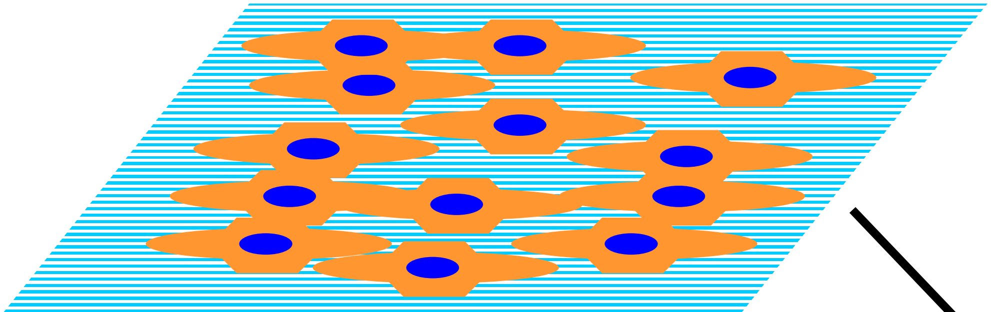


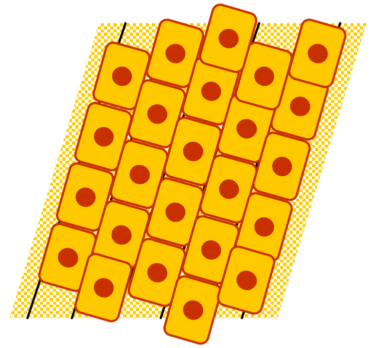

- Tunica adventitia
  - which consist mainly of ***connective tissue fibers***.
- Tunica media
  - formed by a layer of ***circumferential smooth muscle*** and variable amounts of connective tissue.
- Tunica intima
  - comprises its ***endothelial lining*** (typically simple, squamous) and associated connective tissue.

**School of Anatomy and Human Biology - The University of Western Australia**
**http://www.lab.anhb.uwa.edu.au/mb140/CorePages/Vascular/Vascular.htm**

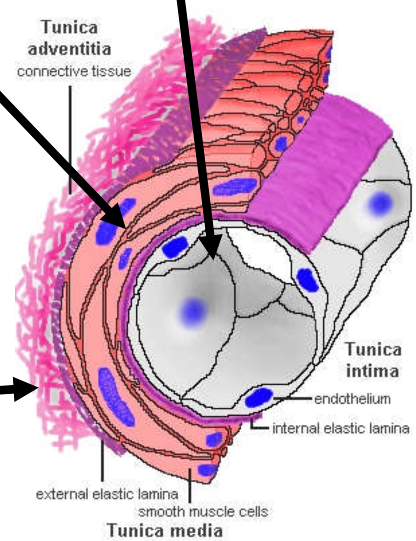
# Blood Vessel Tissue Engineering

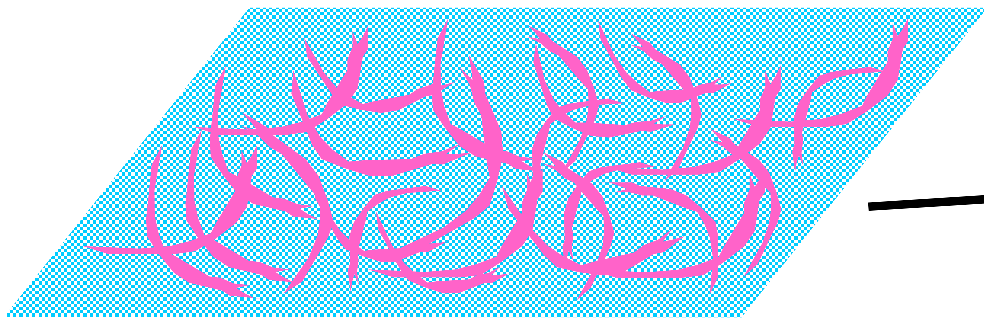
**Smooth muscle cells**
**+**
**aligned nanofibers**





**Endothelial cells**
**+**
**aligned nanofibers**



**Fibroblast cells**
**+**
**Random nanofibers**

# Conclusions

NUS National University of Singapore

**Electrospun polymer nanofibers have tremendous potential in medicine, biotechnology and engineering. To realize this potential further progress is needed in the following areas:**

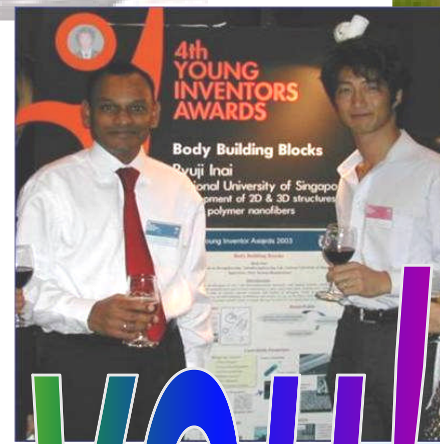
- **Ability to produce nanofibers with controlled dimensions and place them in desired patterns & assemblies reproducibly.**
- **Scale-up the process to industrial level cost effectively.**
- **Ability to measure, predict, tailor and control physical, mechanical, chemical, biological, optical and electrical properties of nanofibers.**

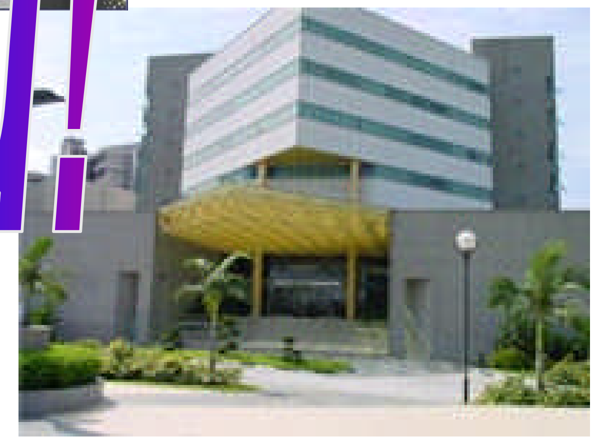
# National University of Singapore Snapshots

NUS
National University of Singapore







Thank you!