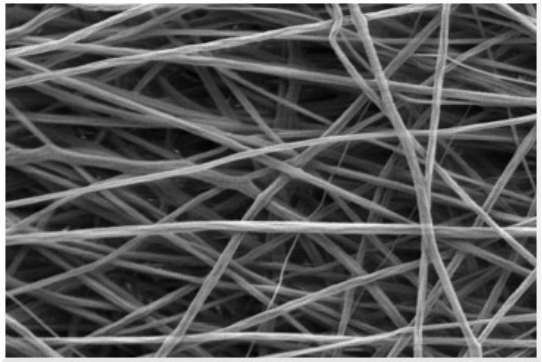
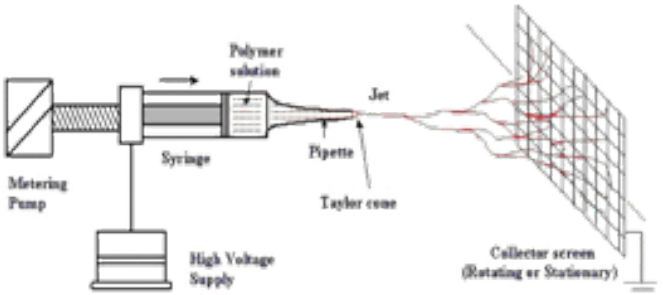
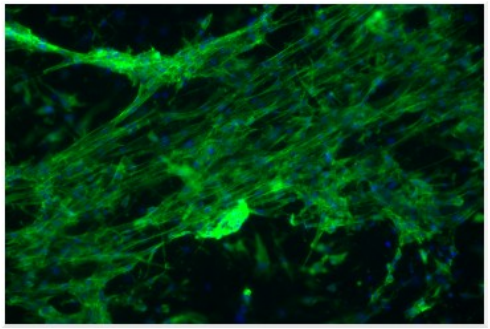
DEVELOPMENT OF POLYMER NANOCOMPOSITE SCAFFOLDS FOR TISSUE ENGINEERING







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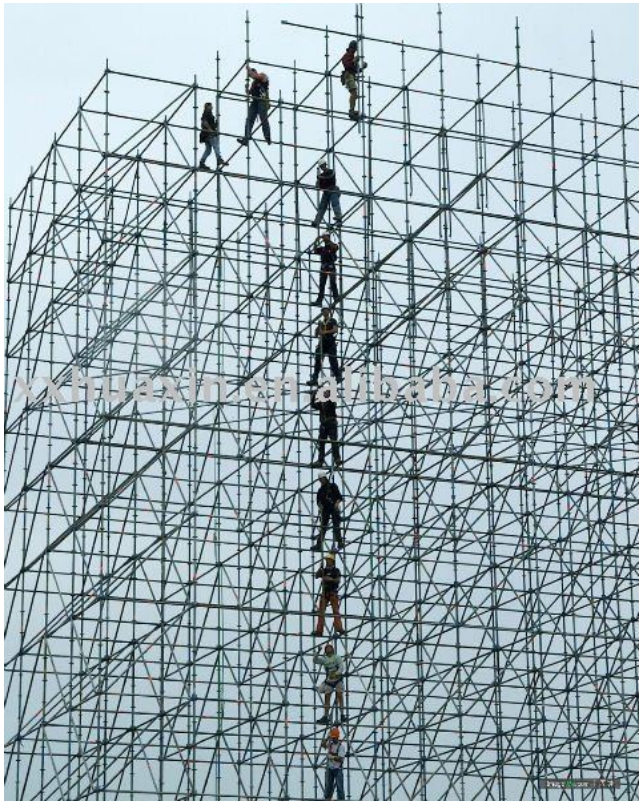
Mahatma Gandhi University

Kottayam, India 686 560, www.iiucnn.ac.in

SCAFFOLD

A temporary platform, either supported from below or suspended from above, on which workers sit or stand when performing tasks at heights above the ground . It consists of one or more wooden planks and is supported by either a timber or a tubular steel or aluminium frame.

To provide or support with a raised framework or platform.



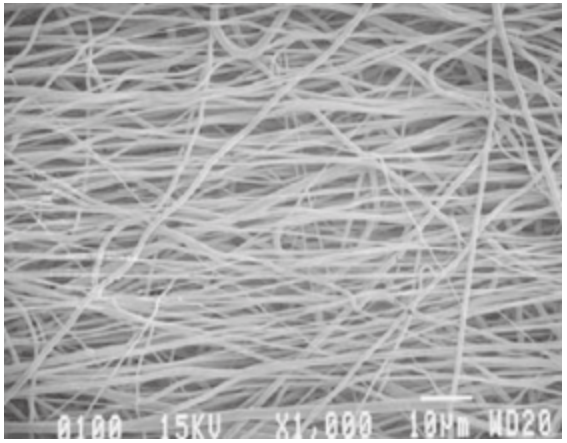
SCAFFOLDS IN TISSUE ENGINEERING

- Provide a platform for cell function, adhesion and transplantation
- It functions as a template to allow new tissue growth and also provides temporary structural support.
- Allow cell attachment and migration
- Deliver and retain cells and biochemical factors
- Enable diffusion of vital cell nutrients
- Exert certain mechanical and biological influences to modify the behaviour of the cell phase

REQUIREMENTS OF SCAFFOLDS

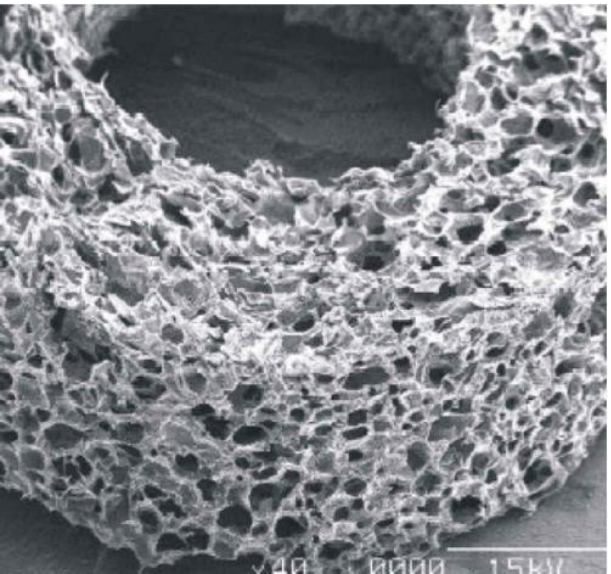
- Good biocompatibility with surrounding tissue
- Large porosity and pore size
- Well interconnected pore network structure for cell adhesion and growth.
- Biodegradability
- Mechanical properties should be similar to the tissue being replaced.
- Diffusability throughout the matrix.
- Provide good attachment with the cells.

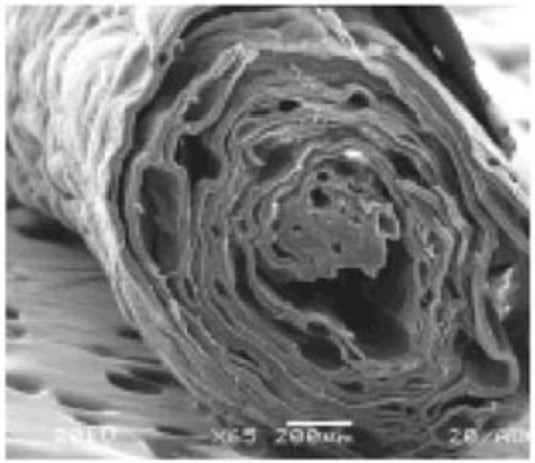
Scaffolds

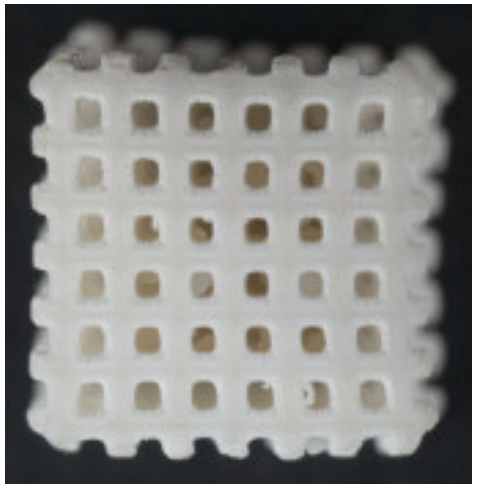


Various textures and materials

- Encourage cells to grow
- Allow nutrients to permeate
- Won't harm the patient







Porous Structure Promotes;

- Cell attachment (macro pores)
- Proliferation (macro pores)
- Differentiation (macro pores)
- Provides pathways for biofluids and wastes (nano pores)
- Favors tissue regeneration (macro pores)

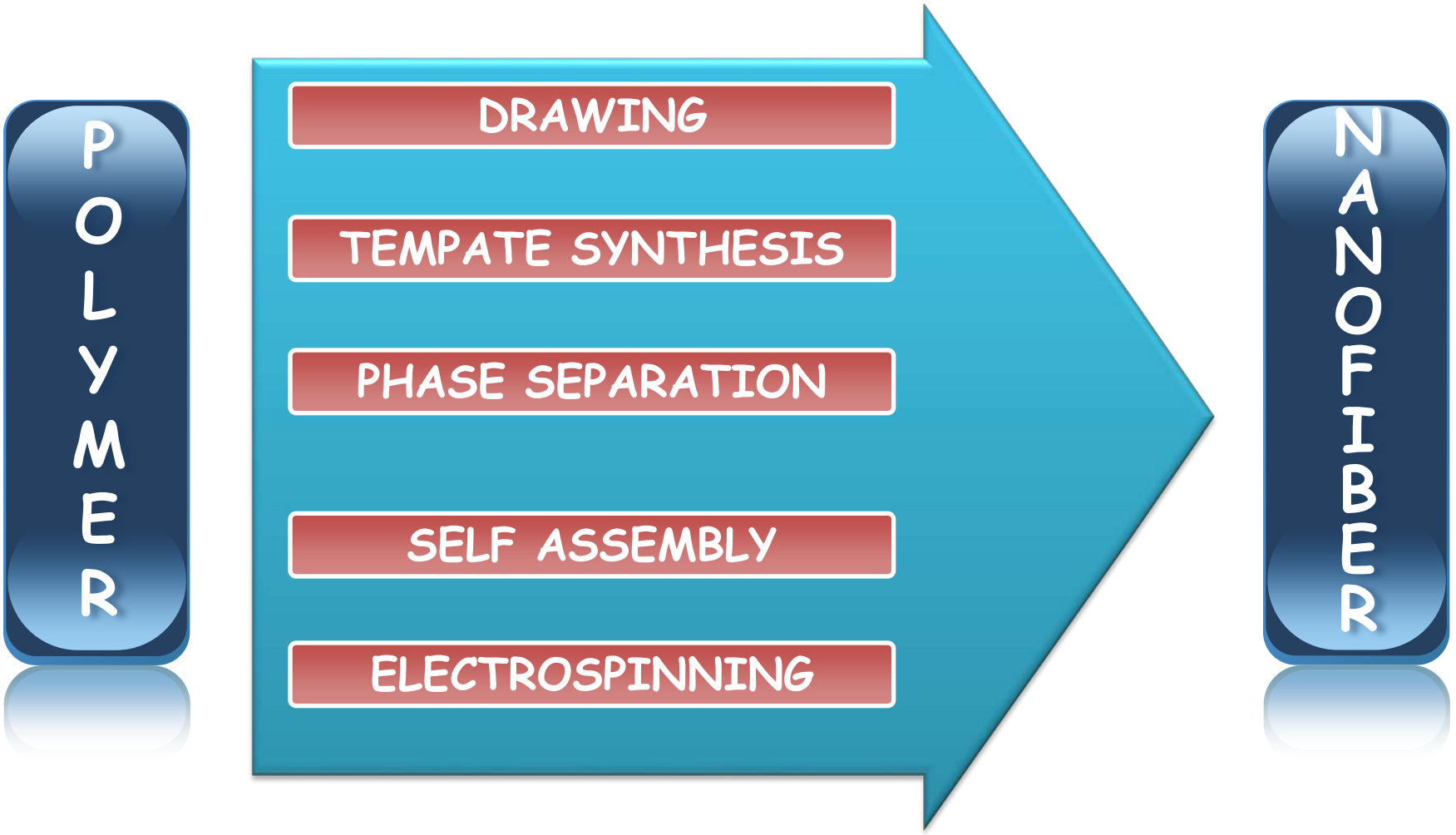
Why do we need fibrous structure??

- Fibers are suitable for use as scaffolding components
  - -compared to other sturctures (eg: particles) due to their continuous structure.

- Advantage of scaffold composed of ultrafine, continuous fibers:

-High porosity.
-Variable pore size distribution.
-High surface –to-volume ratio
- Morphological similarity to natural ECM

Nanofiber Production Techniques



ELECTROSPINNING

• Electrospinning was first observed in 1897 by Rayleigh, studied in detail by Zeleny and patented by Formhals(1934).

• Basically, an electrospinning system consists of three components:
- A high voltage power supply
- A spinneret
- A grounded collector plate

• Both natural as well as synthetic polymers can be electrospun

•Over the years, more than 200 polymers have been eletrospun successfully

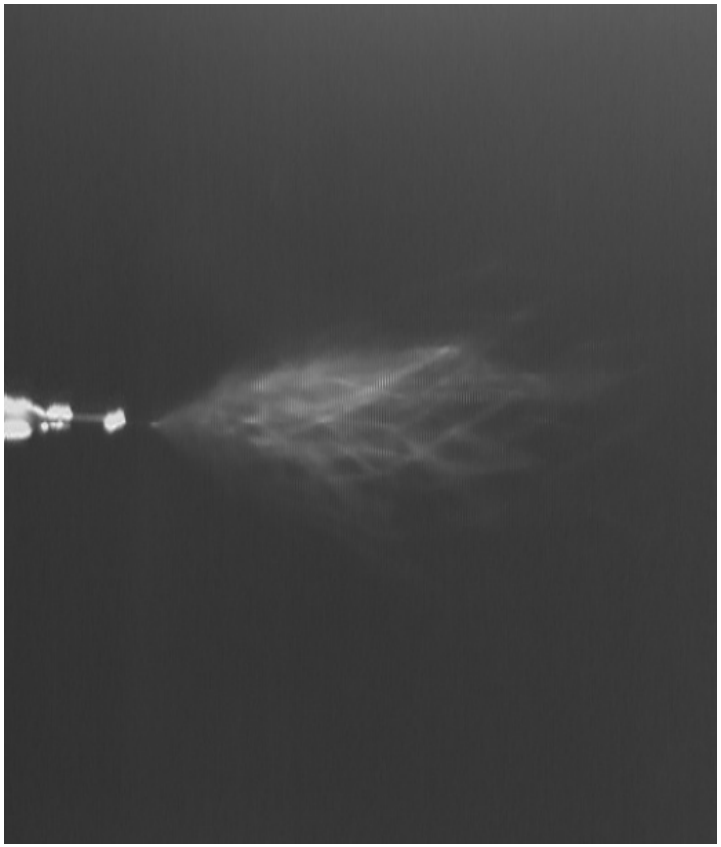
Electrospinning of fibres

Electrospinning, a fiber spinning technique that relies on electrostatic forces to produce fibers in the nanometer scale.

Under the influence of the electrostatic field, a pendant droplet of the polymer solution at the spinneret is deformed into a conical shape.

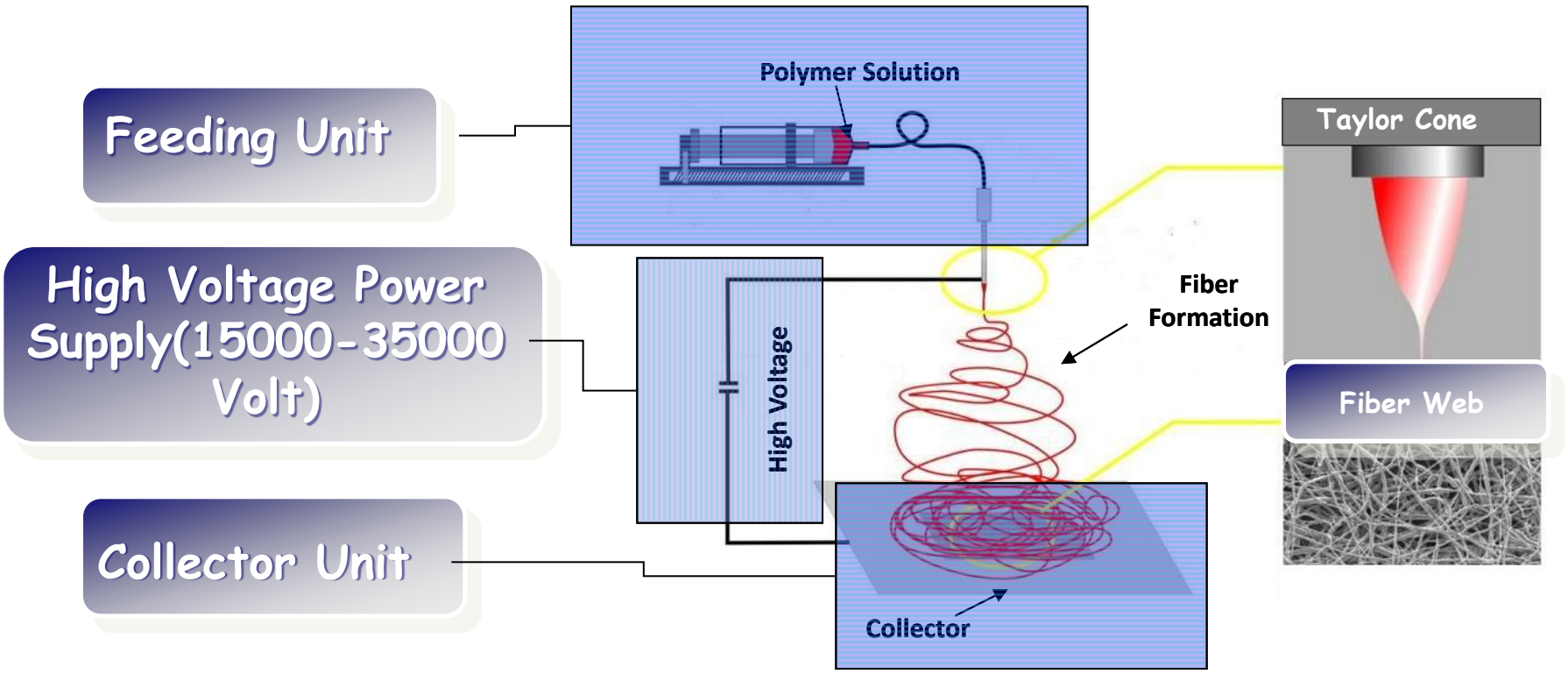
If the voltage surpasses a threshold value, electrostatic forces overcome the surface tension, and a fine charged jet is ejected.

As these electric forces increase, the jet will elongate, accelerate by the electric forces and the solvent evaporates.



Electrospinning Process

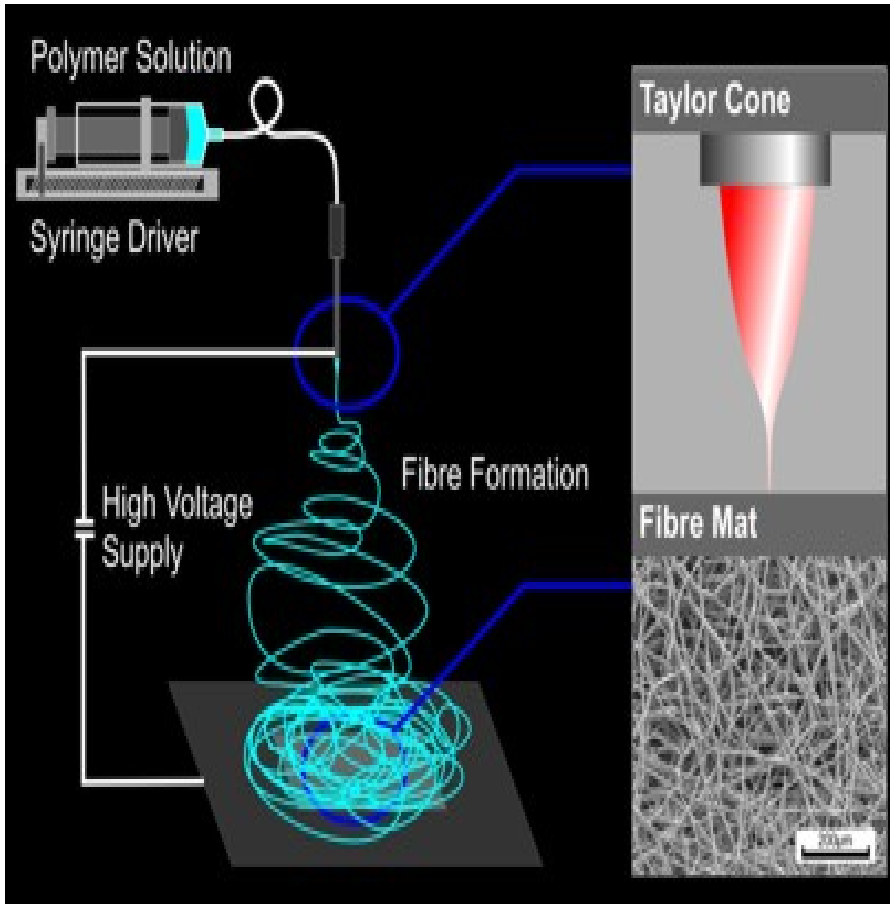
Three Main Components of Electrospinning;



Electrospinning occurs when the electrical forces at the surface of a polymer solution overcome the surface tension and cause an electrically charged jet to be ejected

Electrospinning can be considered as a five step process

- Charging of the solution
- Formation of the cone jet (*Taylor Cone*) when the electrostatic force dominates the surface tension
- Thinning of the steady jet
- Beginning and growth of instabilities resulting the diameter reduction of the fibers into the sub-micron regime & termination thinning of the charged jet
- Collection of the fibers into useful forms



Overview Nanofiber

Process	Ease	Advantages	Limitations
Self-assembly	Difficult	Produce fiber on lowest ECM scale (5-8 nm)	• Lack of control • Limitation on polymers
Phase separation	Easy	• Tailorable mechanical prop. • Batch to batch consistency	•Lab scale production • Limitation on polymers
Electrospinning	Easy	• Cost effective • Long continuous fibers • Tailorable mech properties, size & shape	• Large scale fibers • No control over 3D pore structure

Parameters affecting electrospinning

Electrospinning process is solely governed by many parameters which play a significant role in determining the morphology and diameter of electrospun nanofibers. Parameters can be divided into three.

- Solution parameters
- Operational parameters
- Ambient parameters

Solution Parameters

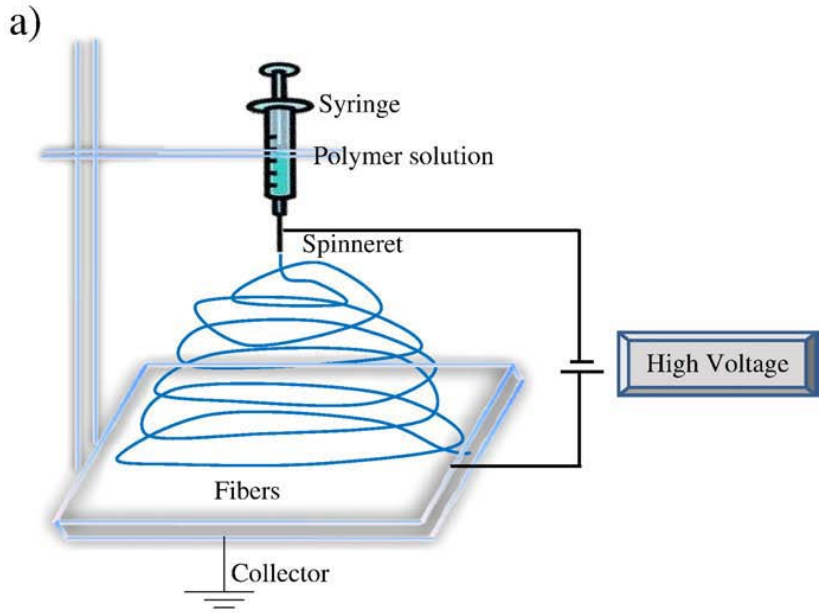
- Molecular weight.
- Concentration /viscosity of the solution
- Surface tension
- Conductivity of the solution

Operational Parameters

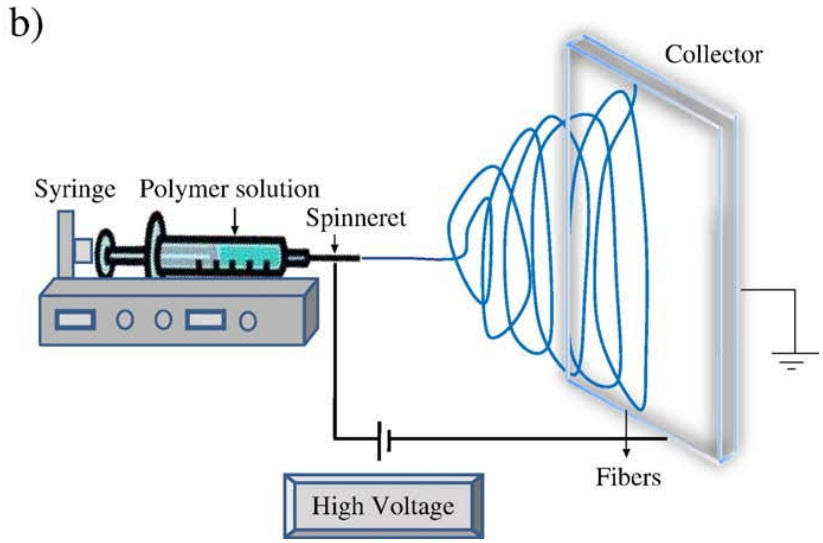
- Applied electric voltage
- TCD (distance between tip and collector)
- Flow rate
- Effect of Collector

Ambient Parameters

- Temperature
- Humidity
- Air Velocity



Vertical set-up



Horizontal set-up

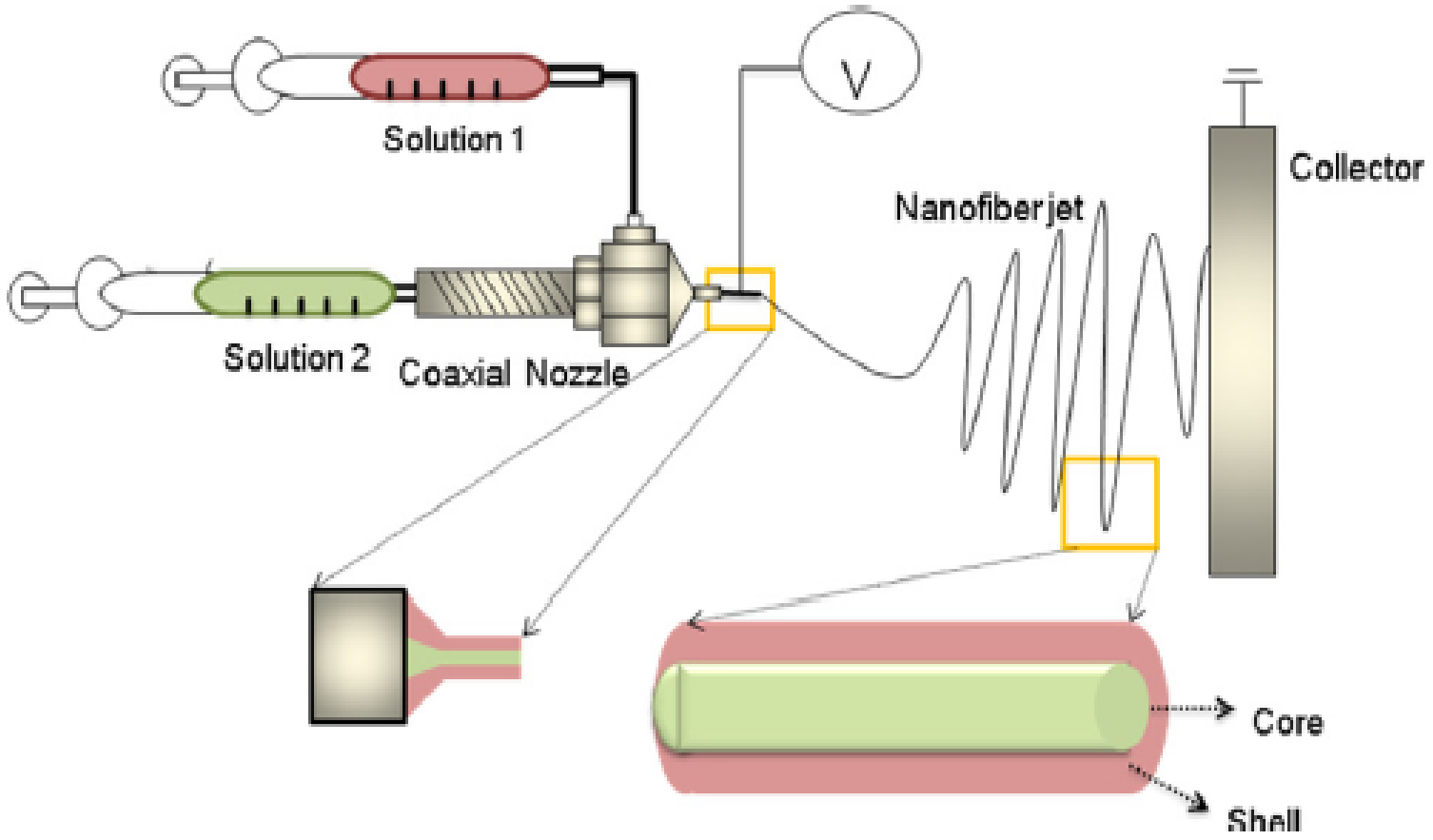


Figure 4. Core–shell nozzle design used to encapsulate drugs within the nanofiber.

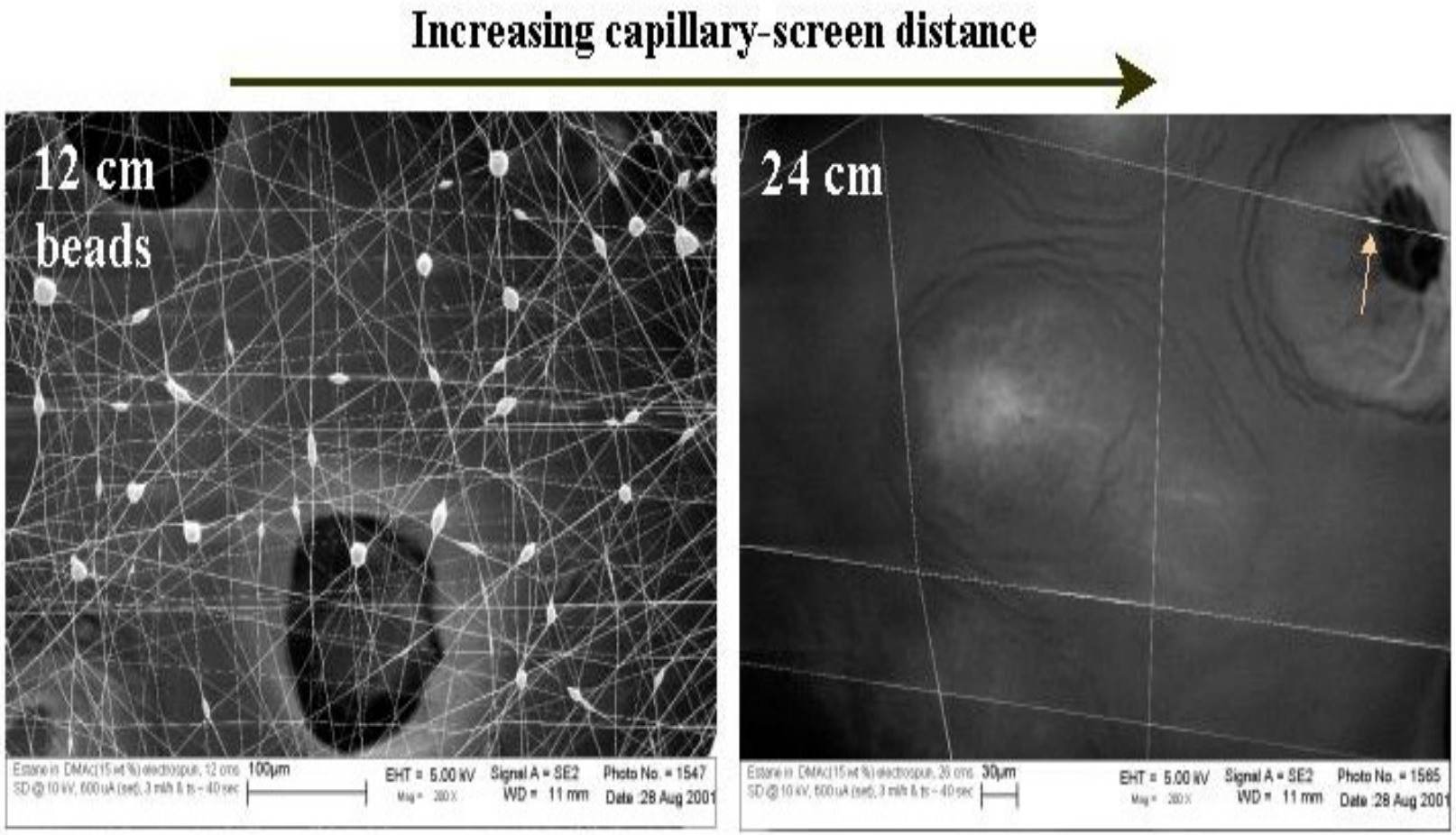


Figure 2. Effect of increasing capillary-screen distance on 15 wt % Estane® 5750 electrospun at 10 kV & 3ml/h. Average diameter range ~ 1 μm - 148 nm & bead size ~ 10 μm - 2.5 mm. The average diameter of fibers & bead-size decreases with increasing capillary-screen distance.

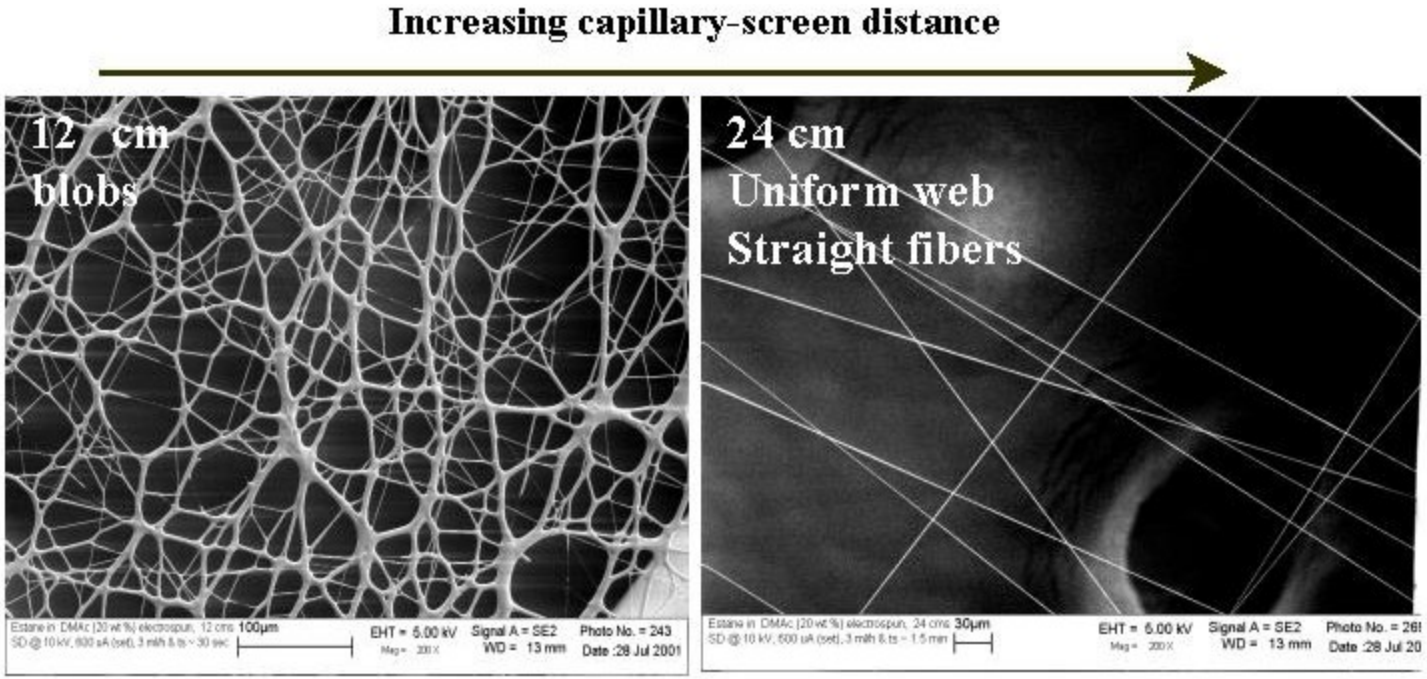


Figure 3. Effect of increasing capillary-screen distance on 20 wt % Estane® 5750 electrospun at 10 kV & 3ml/h. Average diameter range 5 μm – 333 nm. The average diameter of fibers decreases with increasing capillary-screen distance.

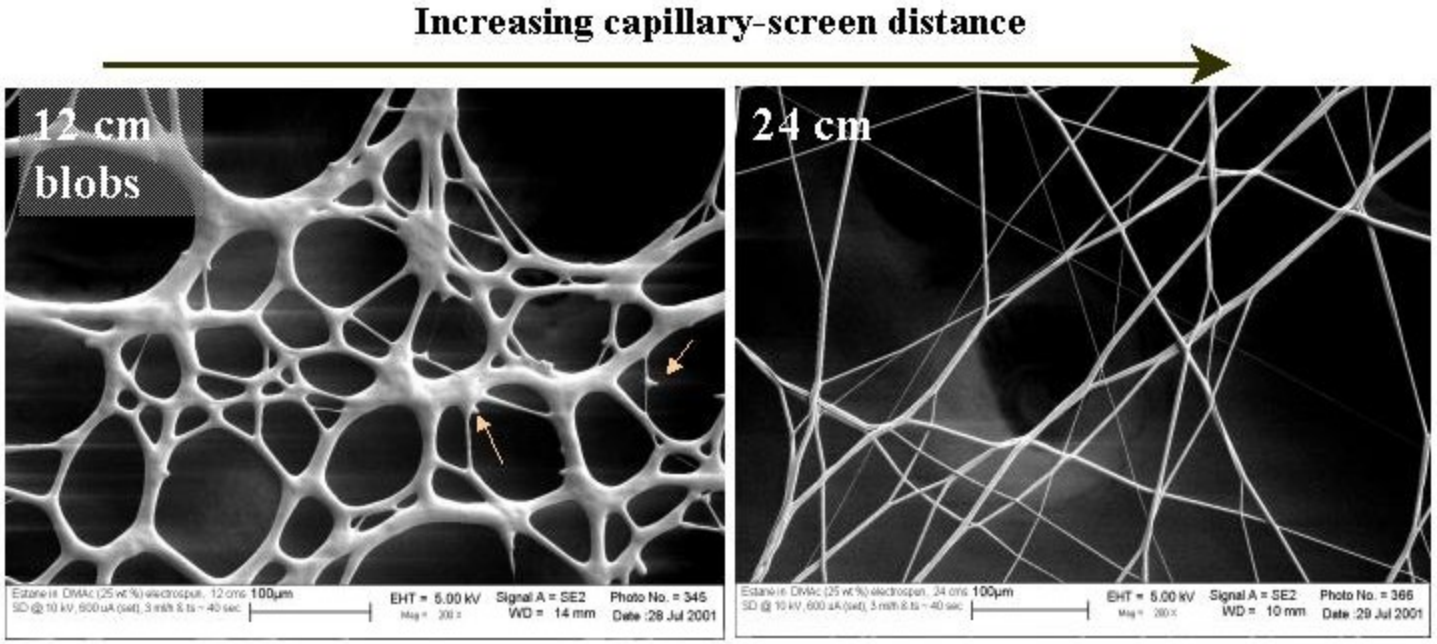


Figure 4. Effect of increasing capillary-screen distance on 25-wt % Estane® 5750 electrospun at 10 kV & 3ml/h. The Average diameter range is 5 μm – 905 nm. A broad distribution of fiber diameters was obtained.

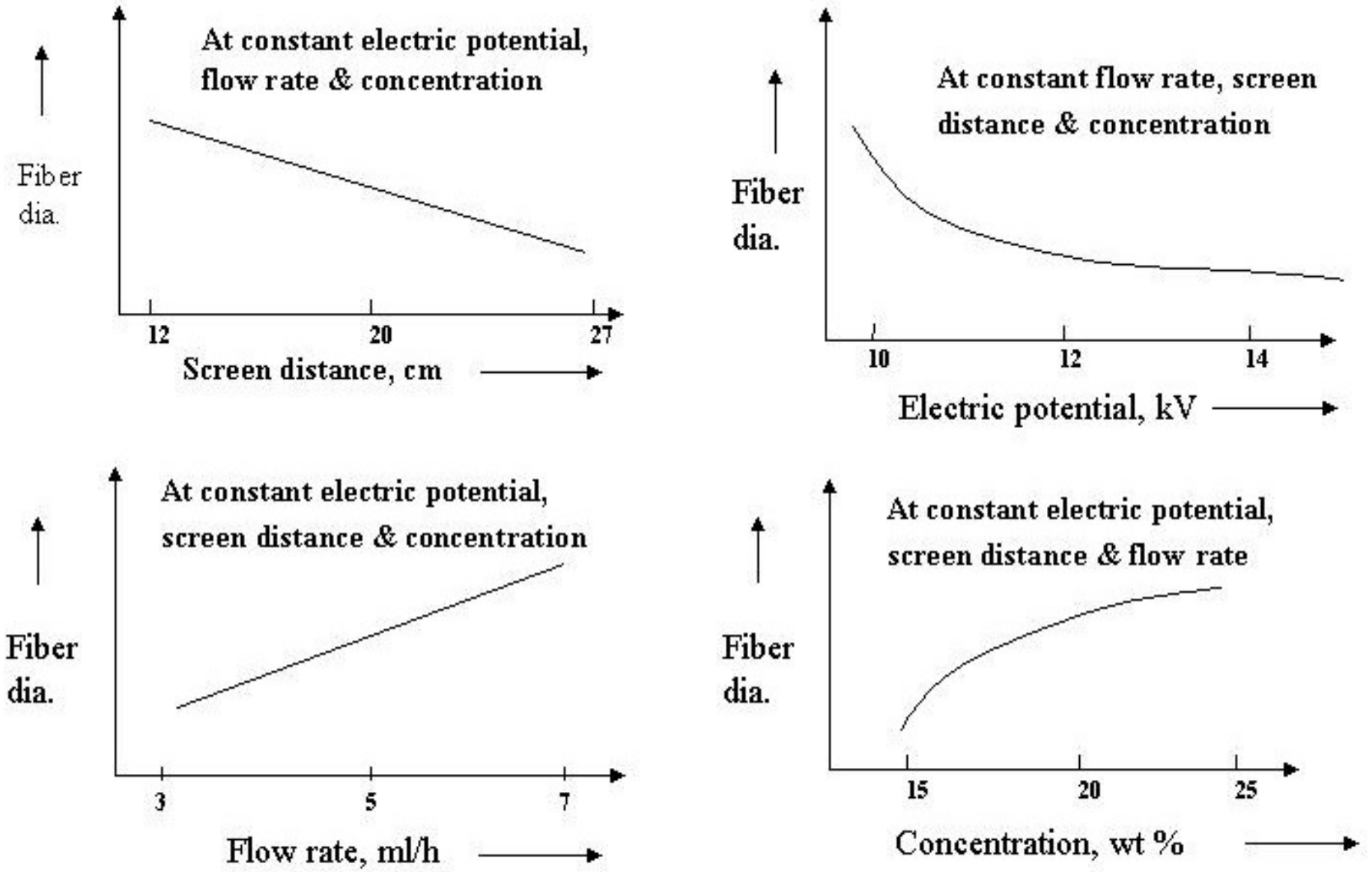


Figure 6. Effect of process parameters on fiber diameter, produced by Electrospinning

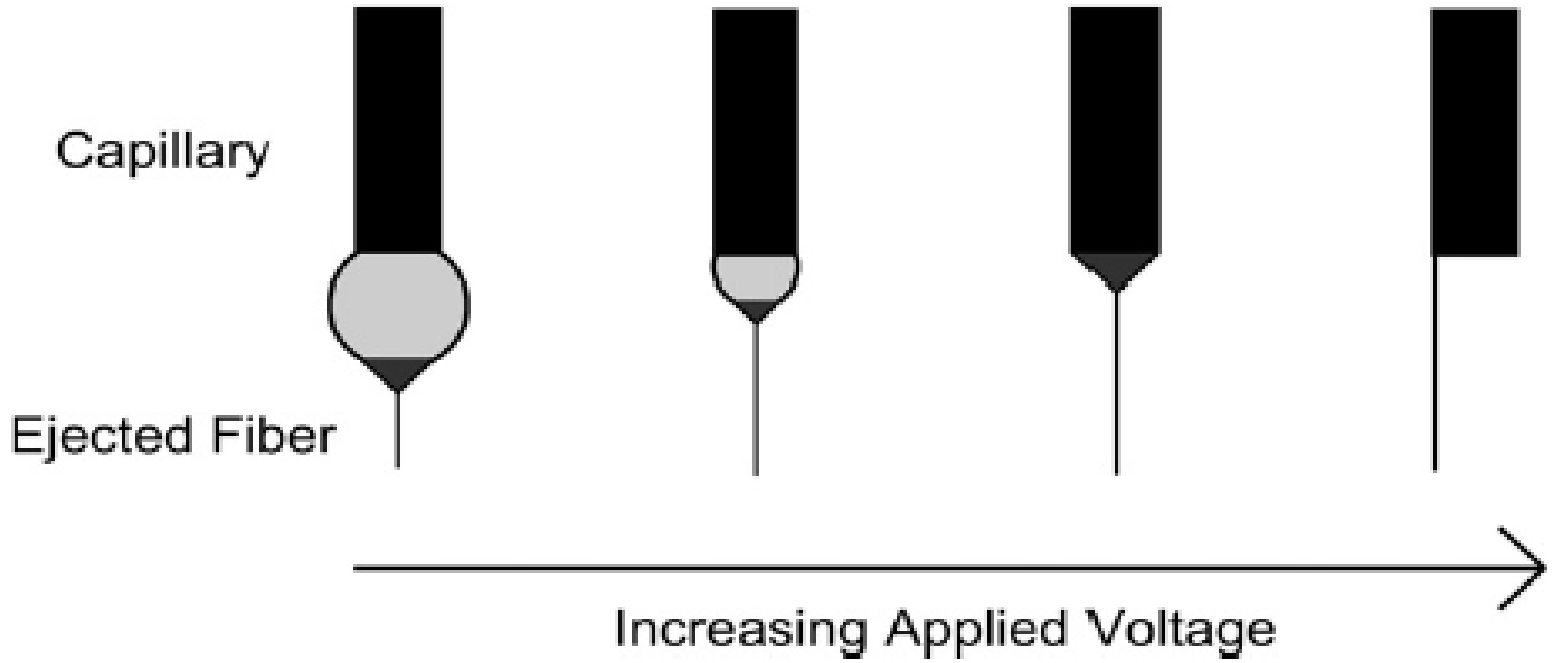
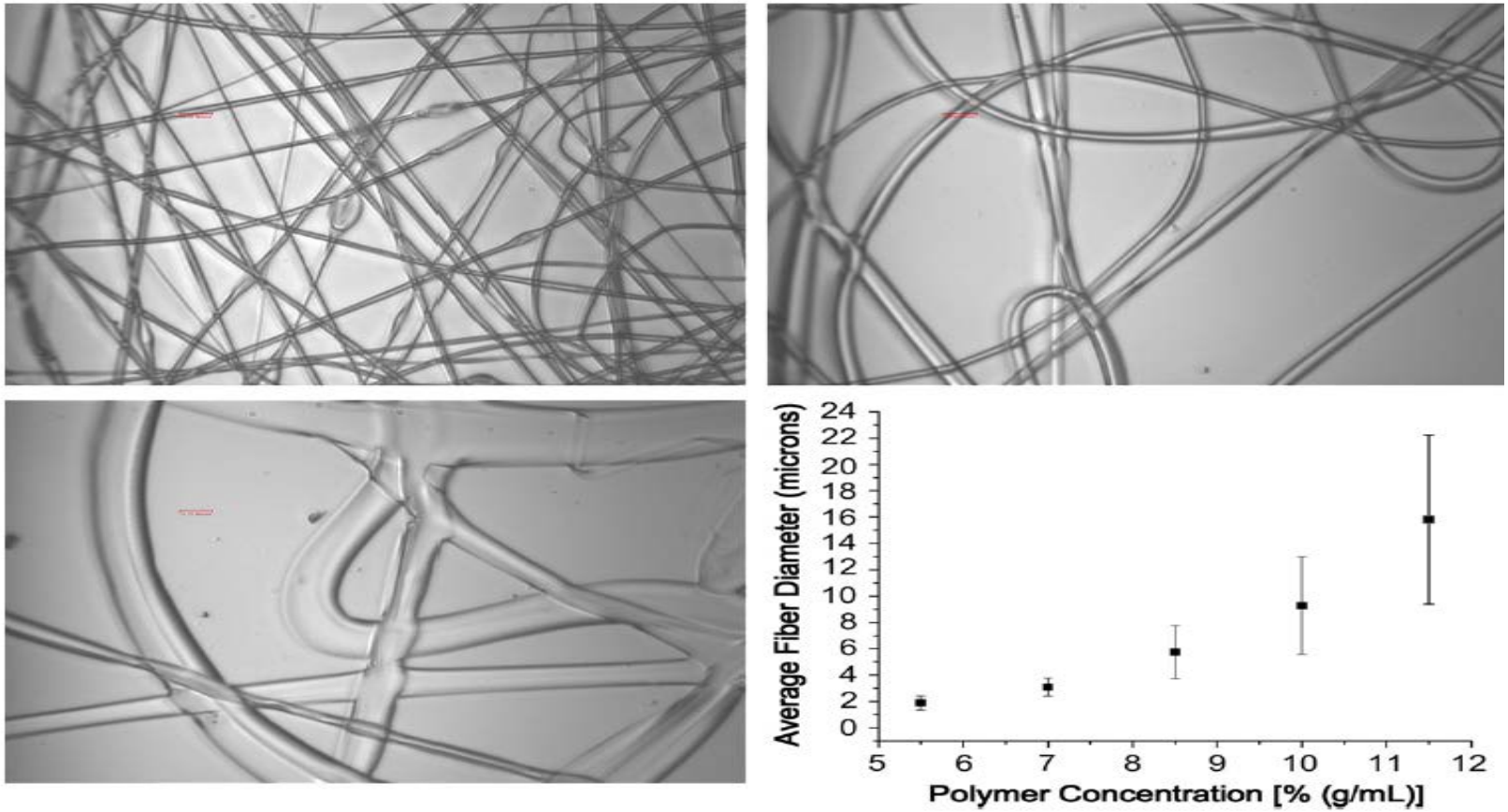


Fig. 2. Effect of varying the applied voltage on the formation of the Taylor cone. At relatively low applied voltages a pendant drop (depicted in light gray) is formed at the tip of the capillary. The Taylor cone (depicted in dark gray) then forms at the tip of the pendant drop. However, as the applied voltage is increased (moving from left to right) the volume of the pendant drop decreases until the Taylor cone is formed at the tip of the capillary. Increasing the applied voltage further results in the fiber jet being ejected from within the capillary, which is associated with an increase in bead defects.

Table 1
Effects of electrospinning parameters on fiber morphology

Parameter	Effect on fiber morphology
Applied voltage ↑	Fiber diameter ↓ initially, then ↑ (not monotonic)
Flow rate ↑	Fiber diameter ↑ (beaded morphologies occur if the flow rate is too high)
Distance between capillary and collector ↑	Fiber diameter ↓ (beaded morphologies occur if the distance between the capillary and collector is too short)
Polymer concentration (viscosity) ↑	Fiber diameter ↑ (within optimal range)
Solution conductivity ↑	Fiber diameter ↓ (broad diameter distribution)
Solvent volatility ↑	Fibers exhibit microtexture (pores on their surfaces, which increase surface area)



Effect of polymer concentration on fiber diameter. Fibers were electrospun from solutions containing varying concentrations of poly(ethylene-co-vinyl alcohol)
in 70:30 (v:v) 2-propanol

Polymers used in electrospinning

There are a wide range of polymers that used in electrospinning and are able to form fine nanofibers within the submicron range and used for varied applications. Electrospun nanofibers have been reported as being from various synthetic polymers, natural polymers or a blend of both including proteins, nucleic acids and even polysaccharides.

Natural polymers	Synthetic polymers
Silk fibroin	Poly Lactic acid
Chitosan	Poly caprolactam
Collagen	Poly glycolic acid
Gelatin	Poly urethane
Fibrinogen	Poly (lactide-co-glycolide)

Polymer Nanocomposite Scaffolds

Polymer Nanocomposite Scaffolds appear most promising Biomaterial for Tissue Engineering Applications

- Controlled size
- Surface morphology
- Porosity and Diffusive permeability
- Superior mechanical properties
- Improved durability
- Surface bioactivity
- Biocompatibility
- Biodegradability

} compared with conventional polymers or composites

Polymer Nanocomposites

- Polymers comprising particles at least one dimension in the nanosize range (1-100 nm)
- Class of materials that have properties with significant commercial potential

Attractive features identified with nanocomposites are:

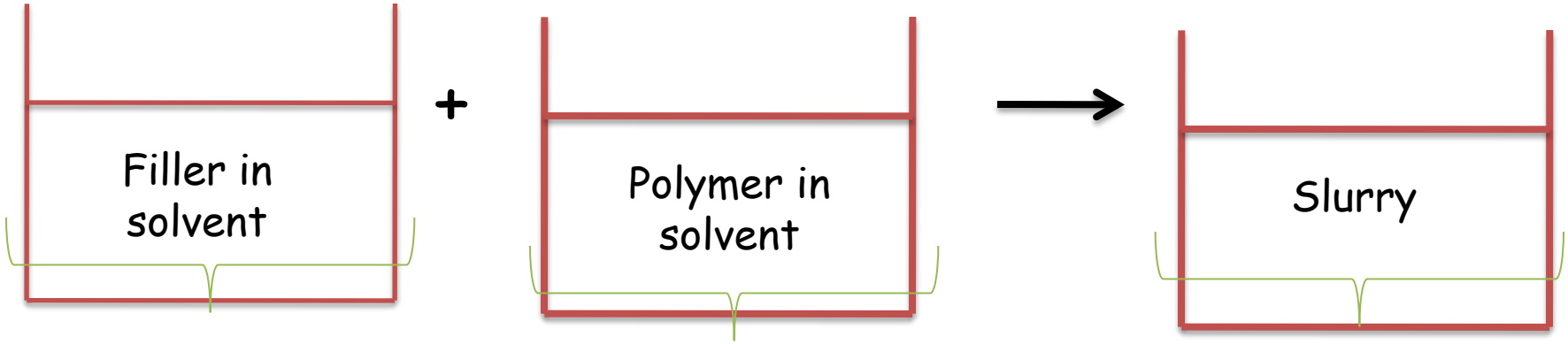
- ✓ Efficient reinforcement without loss of ductility and even improvement in impact strength
- ✓ Excellent optical and altered electronic properties
- ✓ Heat Stability
- ✓ Flame resistance
- ✓ Improved gas barrier properties
- ✓ Improved abrasion resistance
- ✓ Reduced shrinkage and residual stress

Potential Nanocomposite Materials

- Nanotubes
- Graphitic platelets
- Nano talc
- Synthetic and natural clays
- Cellulose fibres (flax, hemp…)
- Metal oxides (TiO2, ZnO), Phosphates

Work done at Mahatma Gandhi University

Various polymer nanocomposite scaffolds are prepared by electrospinning, characterisation is done and extended to relevant applications.



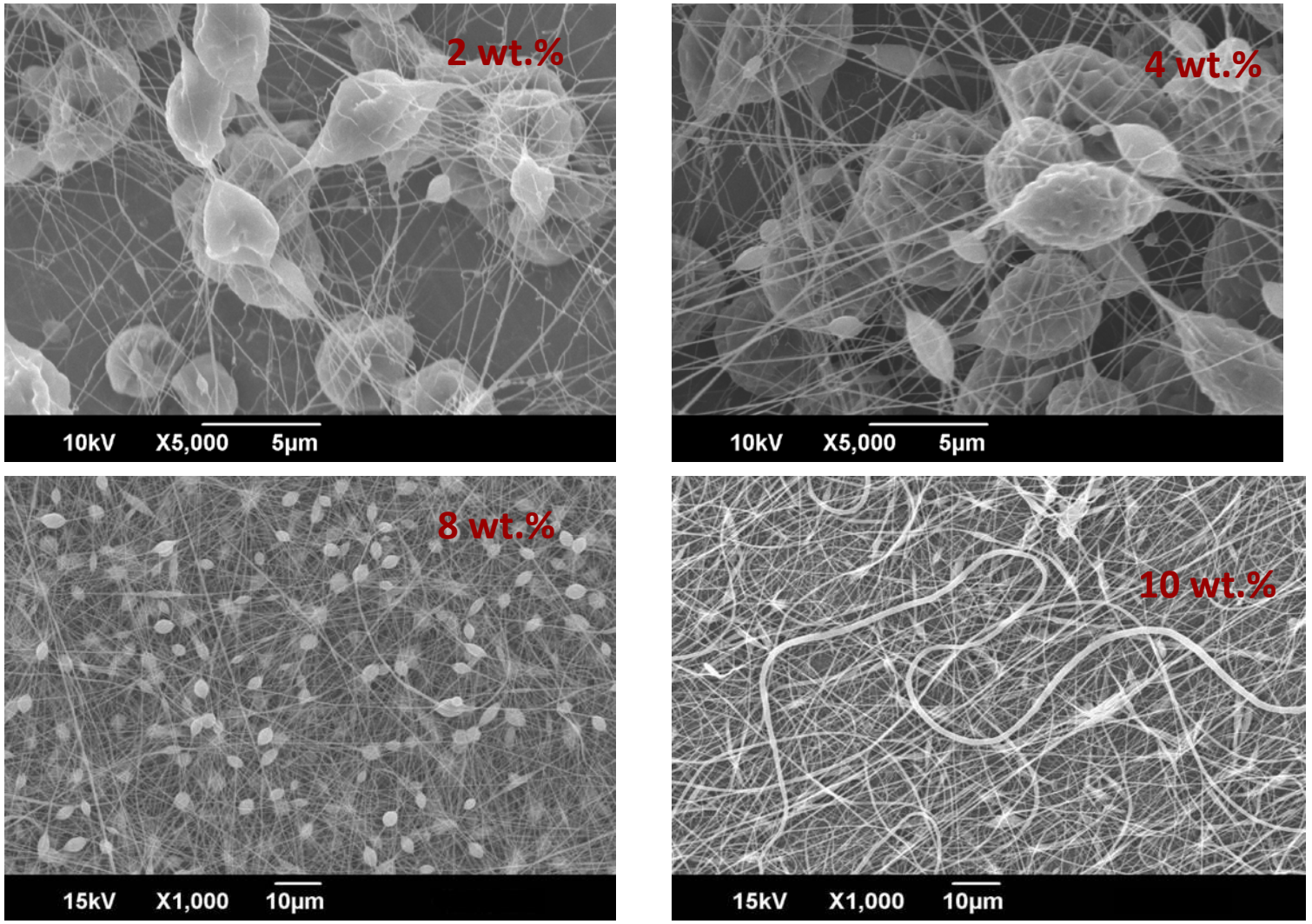
Adjusting ;

- The solution parameters
- operational parameters

✓**Effect of electrospinning processing variables on the membrane structure.**

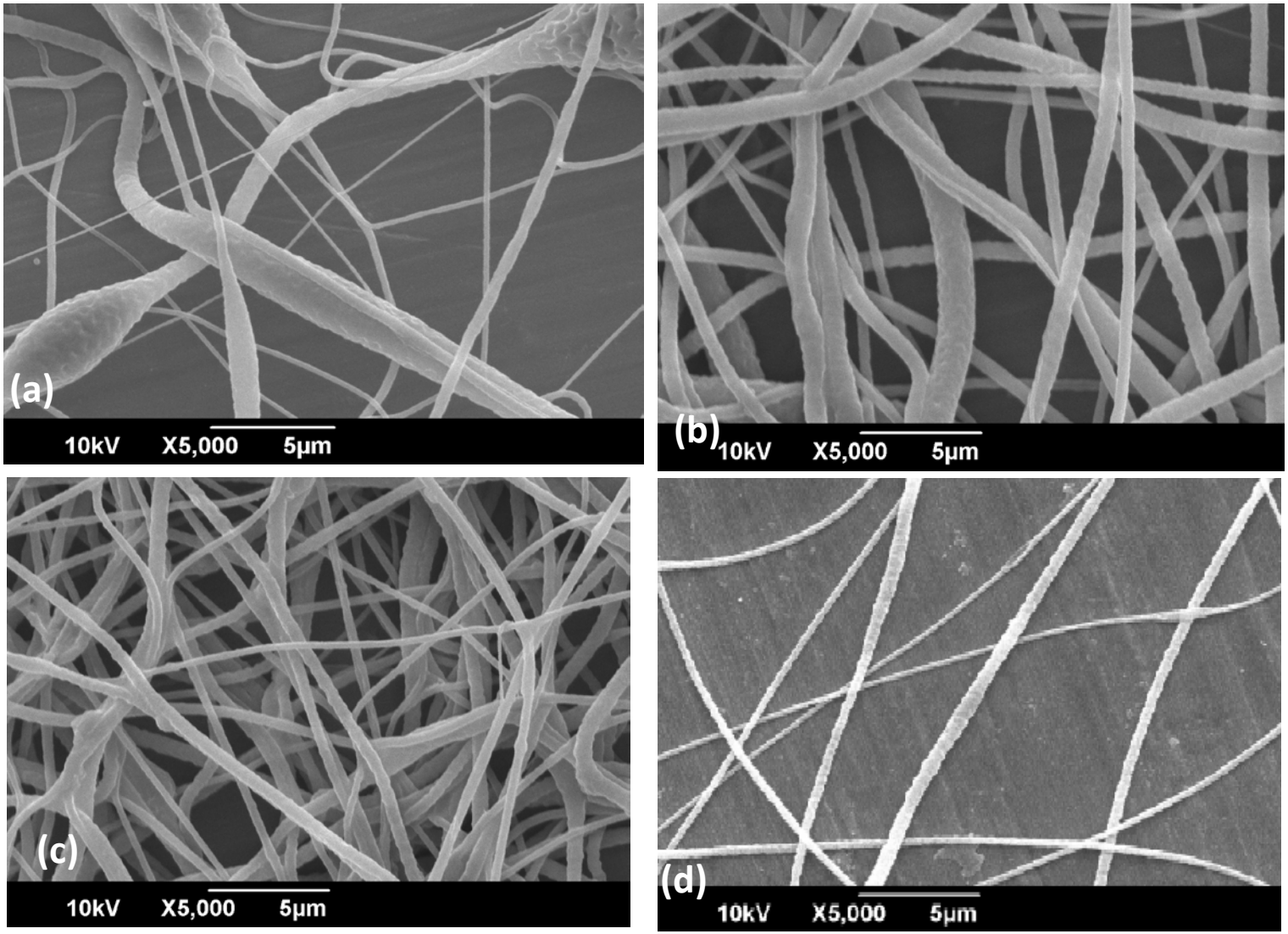
1. Effect of Polymer Concentration
2. Effect of Clay content
3. Effect of Applied Voltage
4. Moisture Content

1. Effect of Polymer Concentration



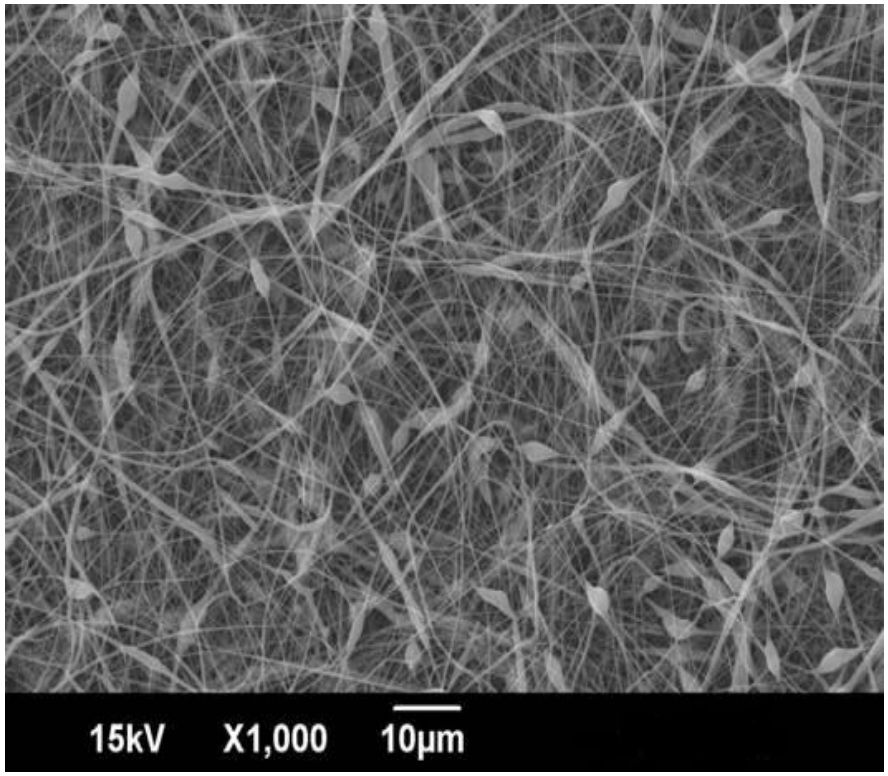
Electrospinning of PCL soln.using DCM solvent (Parameters: Voltage-15 kV, TCD distance-12 cm , Biomacromolecules, submitted

2.Effect of Clay content

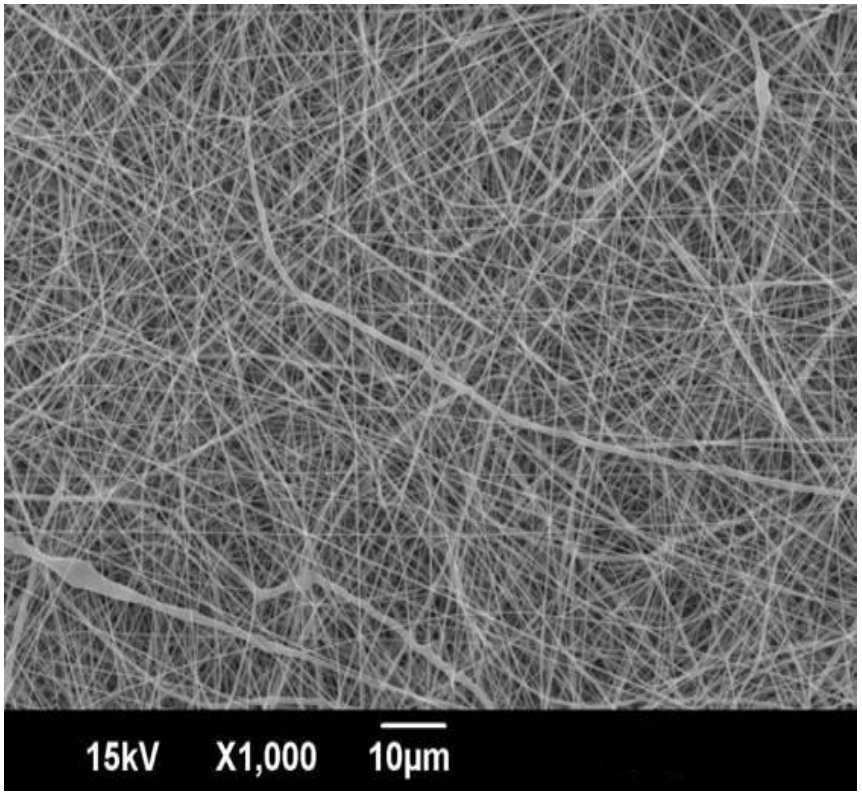


Electrospunfibres of PCL containing (a) 0 wt% Clay,(b) 1 wt%,(C) 5 wt% and (d) 9 wt% **Biomacromolecules, submitted**

3.Effect of Applied Voltage



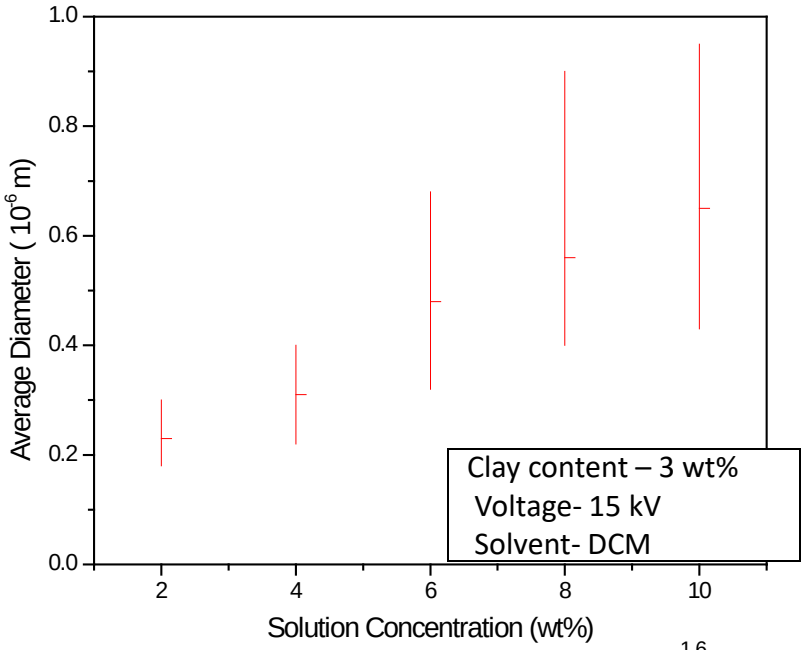
At 8 kV

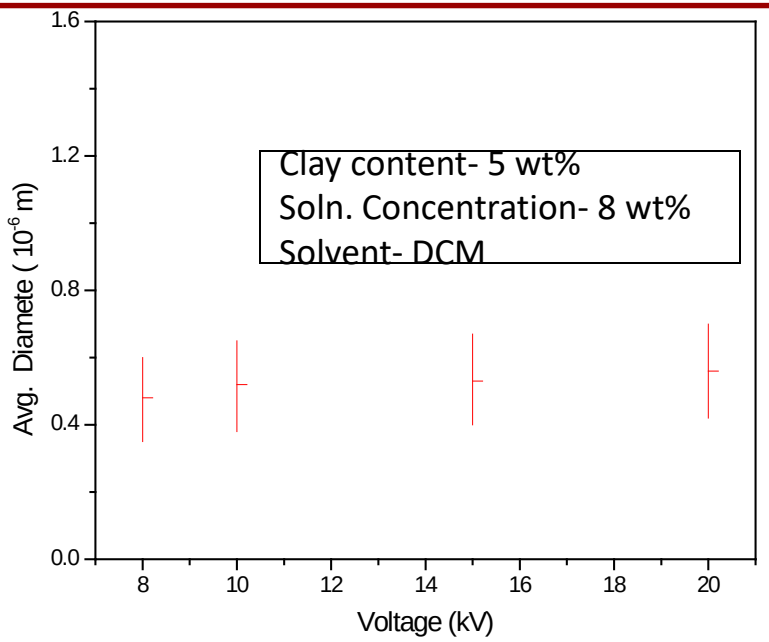


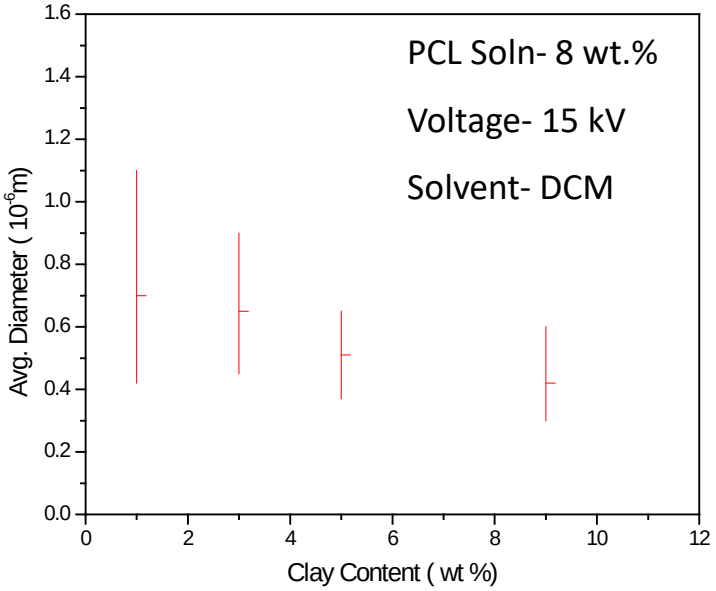
At 15 kV

Electrospinning parameters: PCL soln- 8 wt.%, clay- 5 wt.%, solvent- DCM, Solution feed rate -0.5 ml/hr, Biomacromolecules, submitted

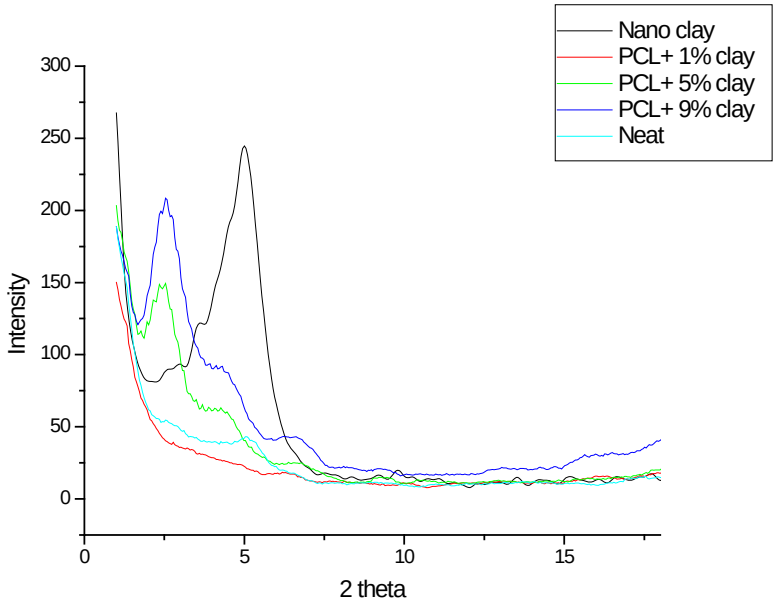
Average Diameter variation with respect to solution concentration, Clay Content and Electrical Potential







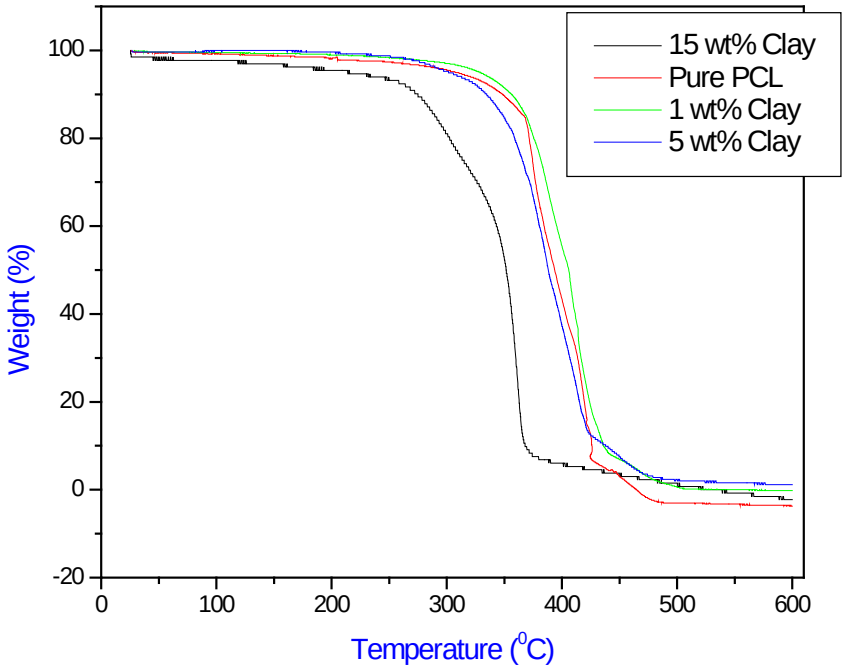
II.XRD Analysis



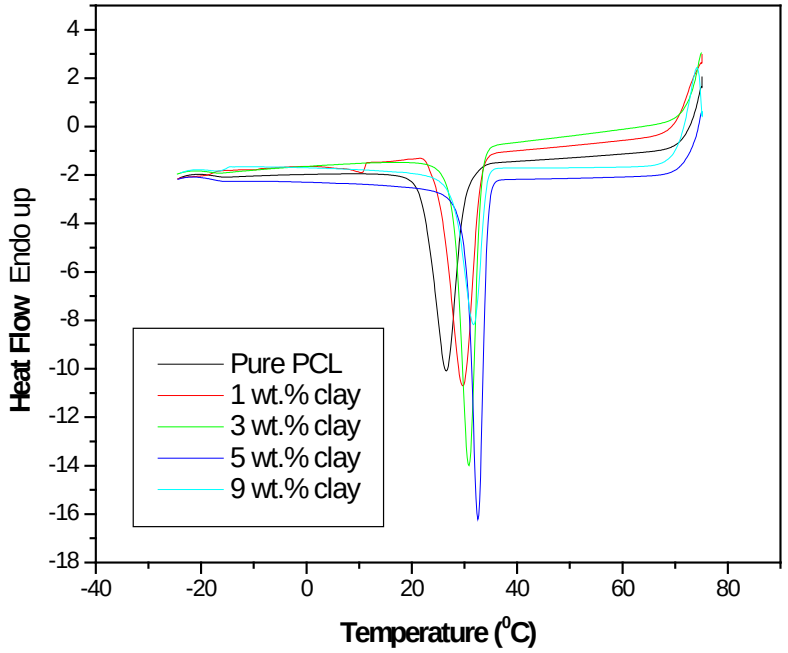
Cloisite 15A,

Biomacromol, submitted

IV.Thermal Analysis



TGA curves of the electrospun fibres and clay



DSC curves of PCL and electrospun fibres,
Biomacromolecules, submitted

Clay **content(wt%)**	T onset (°c)
0	205.4
1	269.2
5	266.2
15	135.7

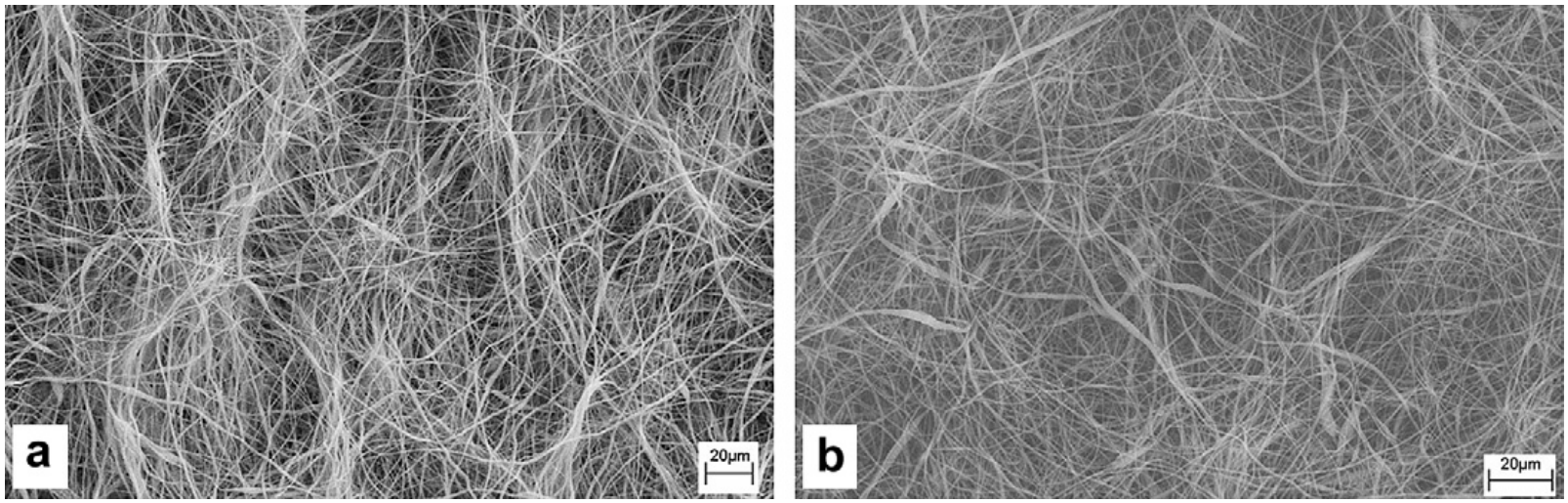
Degree of Crystallanity (%)

Pure PCL	**91.9**
3 wt.% clay	**82.4**
5 wt.% clay	**76.8**
9 wt.% clay	**66.1**

Crystallization Temperature (°C)

Pure PCL	**26.53**
1 wt.% clay	**29.76**
3 wt.% clay	**31.04**
5 wt.% clay	**32.50**
9 wt.% clay	**32.10**

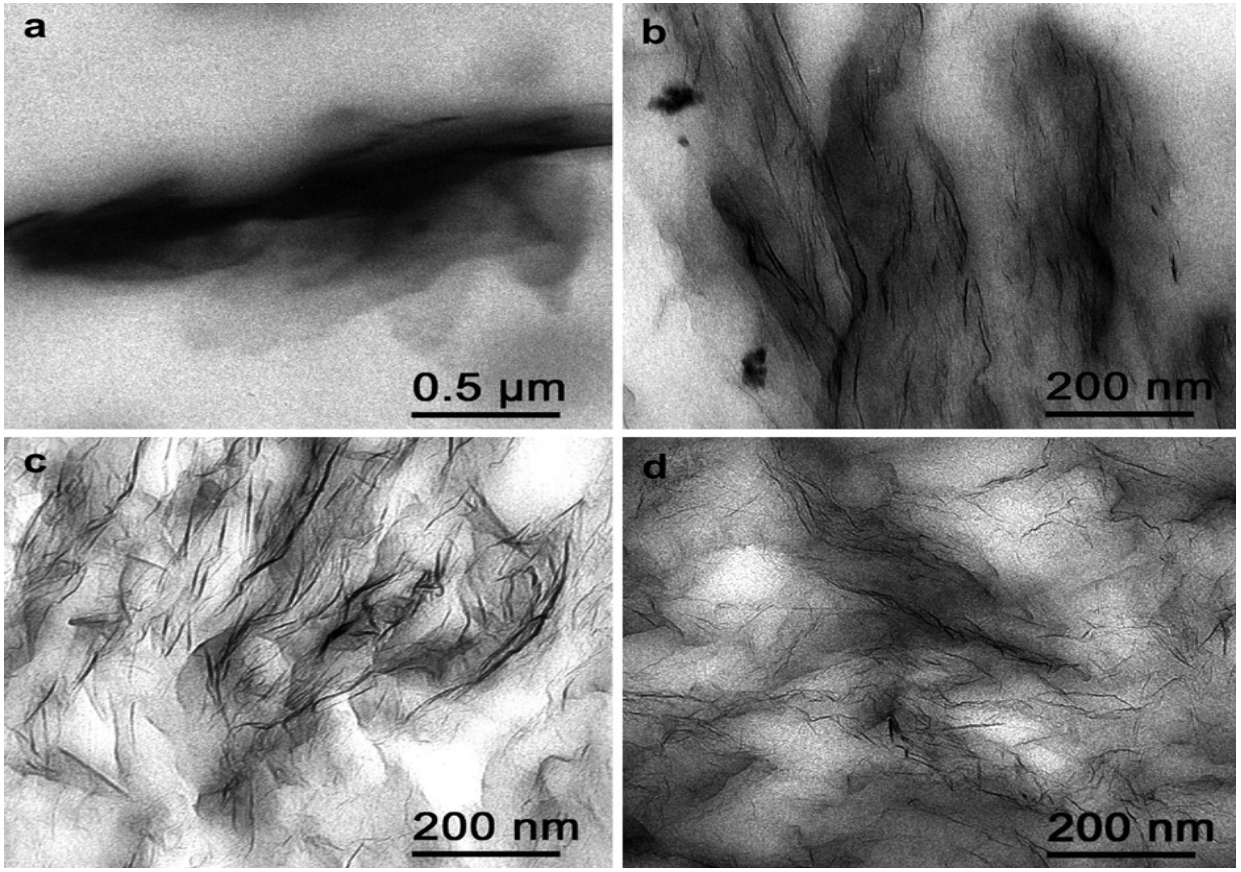
Scanning Electron Microscopy



SEM micrographs of a neat PCL electrospun mat (a) and PCL/CNFs electrospun mats loaded with 1% wt CNFs.

Bhardwaj etal,Biotech. Adv. 2010

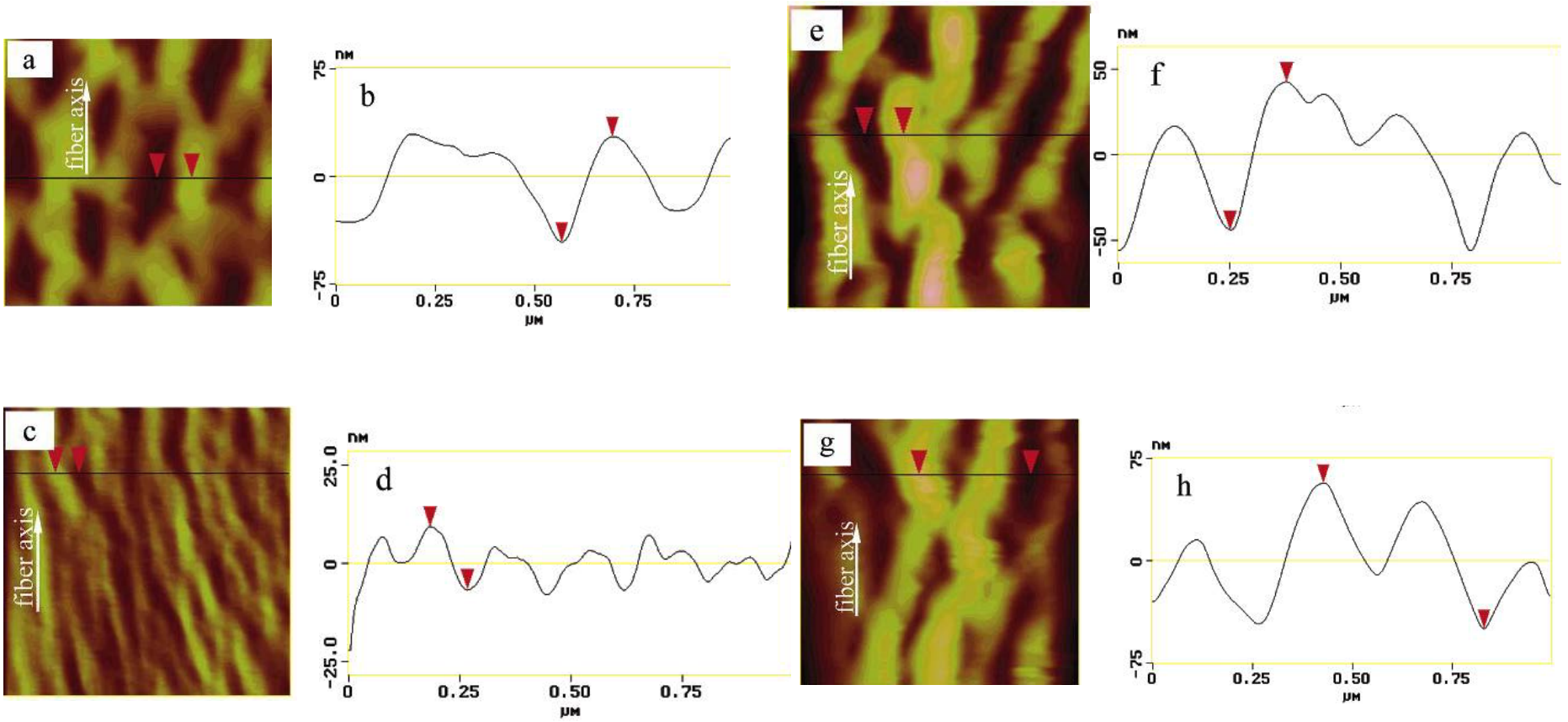
Transmission Electron Microscopy



TEM images illustrating the morphological differences in composites with a thermoplastic poly(urethane) matrix filled with (a) unexfoliated graphite in a stacked morphology, and (b) TEGO, processed by melt mixing. Images (c) and (d) show TEGO/polyurethane composites produced by solution blending and in situ polymerization, respectively, illustrating a more exfoliated state of dispersion

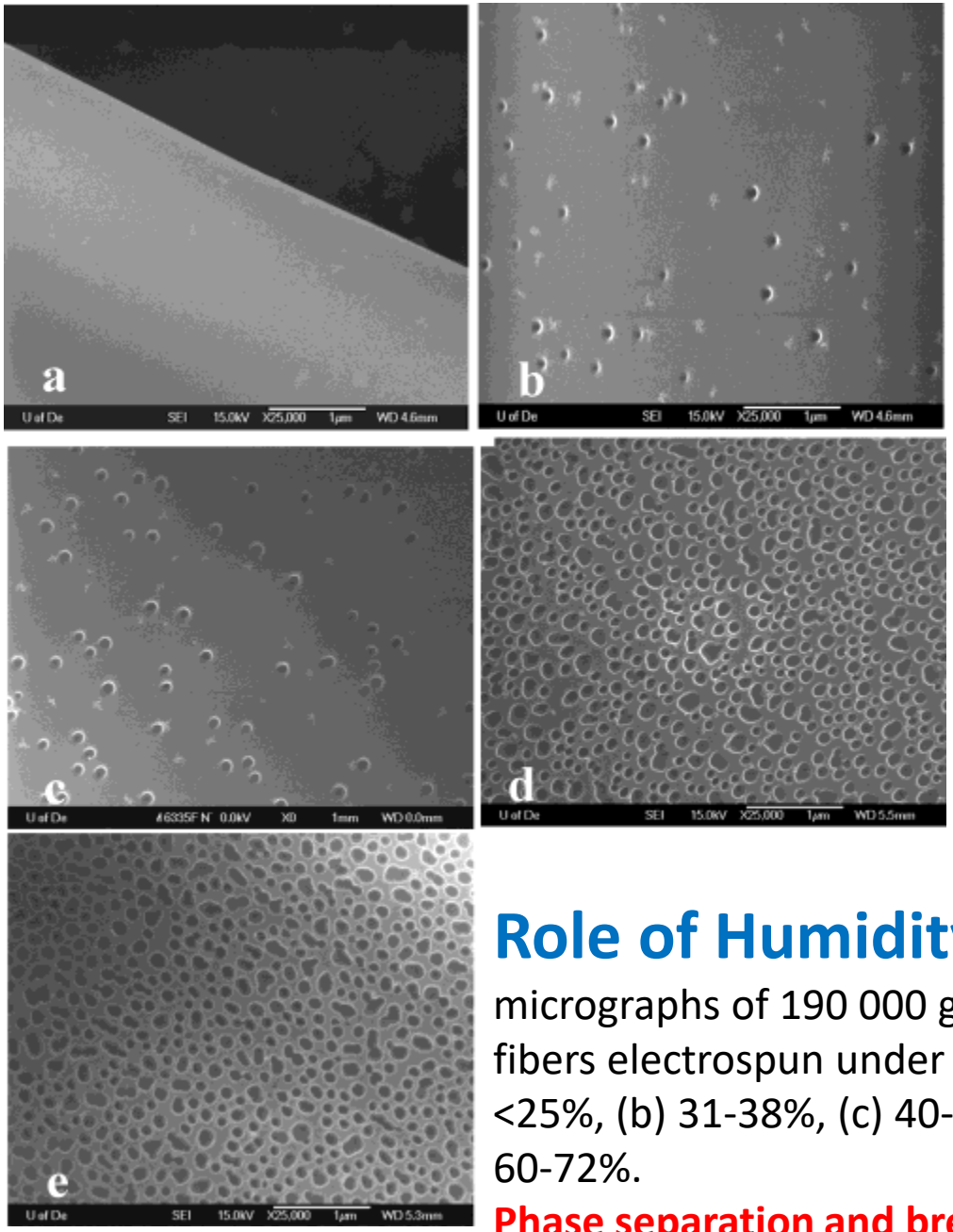
Potts etal, Polymer 2011

AFM



AFM images of surface topography and corresponding cross-sectional profiles of 15 wt % electrospun PS fibres at different clay concentrations (1 x 1μm) *(a) without clay, (c) with 1 wt % clay, (e) with 4 wt % clay, and (g) with 8 wt % clay.*

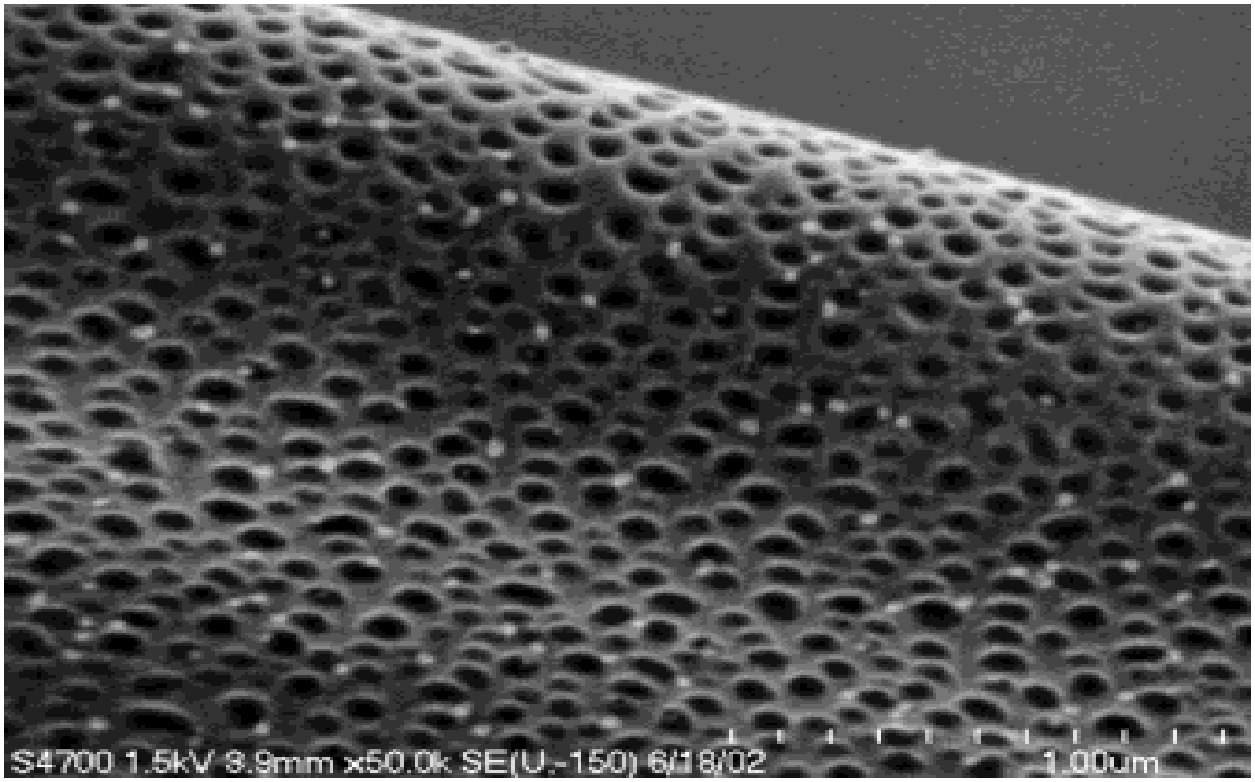
Ji etal, Langmiur, 2006



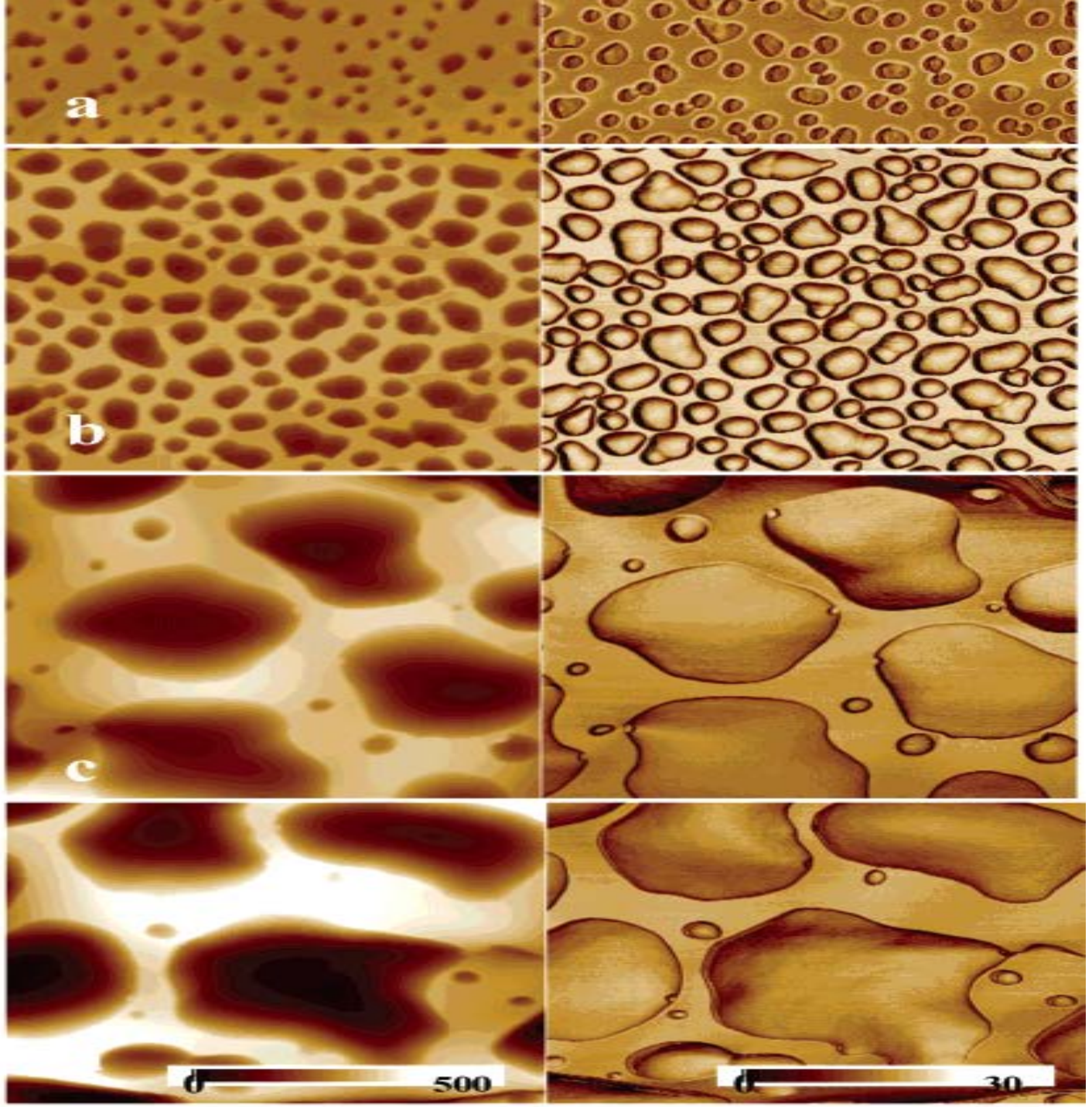
Role of Humidity: FESEM micrographs of 190 000 g/mol PS/THF fibers electrospun under varying humidity: (a) <25%, (b) 31-38%, (c) 40-45%, (d) 50-59%, (e) 60-72%.

Phase separation and breathing.

Macromolecules



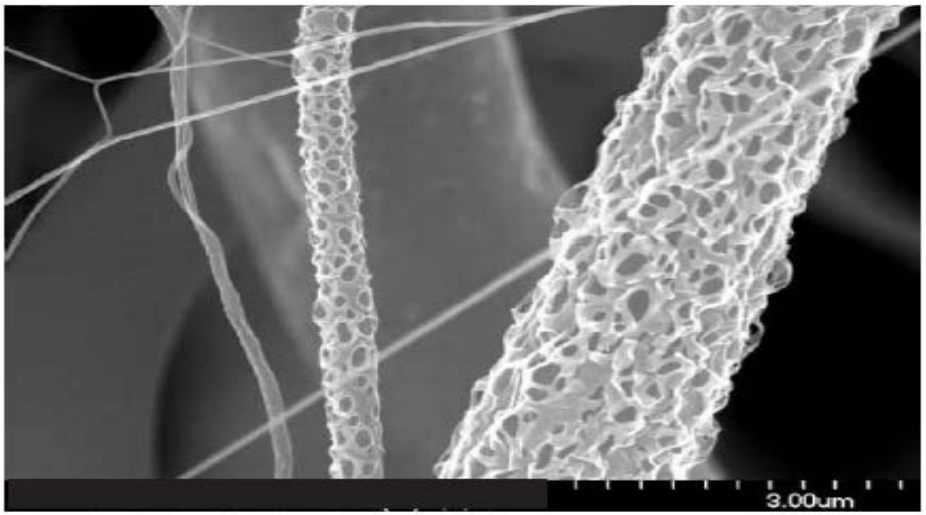
FESEM micrograph of 171 000 g/mol PS/THF fiber electrospun in 50% humidity., Macromolecules


a
b
c
d
500
d
30

- In electrospinning processing, individual fiber diameters typically range from 50 nm to a few micrometers,which necessarily results in a membrane containing pores in the similar range ofelectrospun fibers .The nutrients and metabolic wastes may pass through the nano-sized (ca. 10–1000 nm) porosity ofelectrospun membrane but it seems too small to provide enough space for the cell growth and for the blood vessel invasion. Therefore, it is desirable for the electrospinning technique to be complemented by techniques providing micro-sized (ca. 10–300 mm) porosity and maintaining its robust structure during cell growth and biodegradation

DUAL POROUS SCAFFOLDS PREPARED BY ELECTROSPINNING AND SALT LEACHING

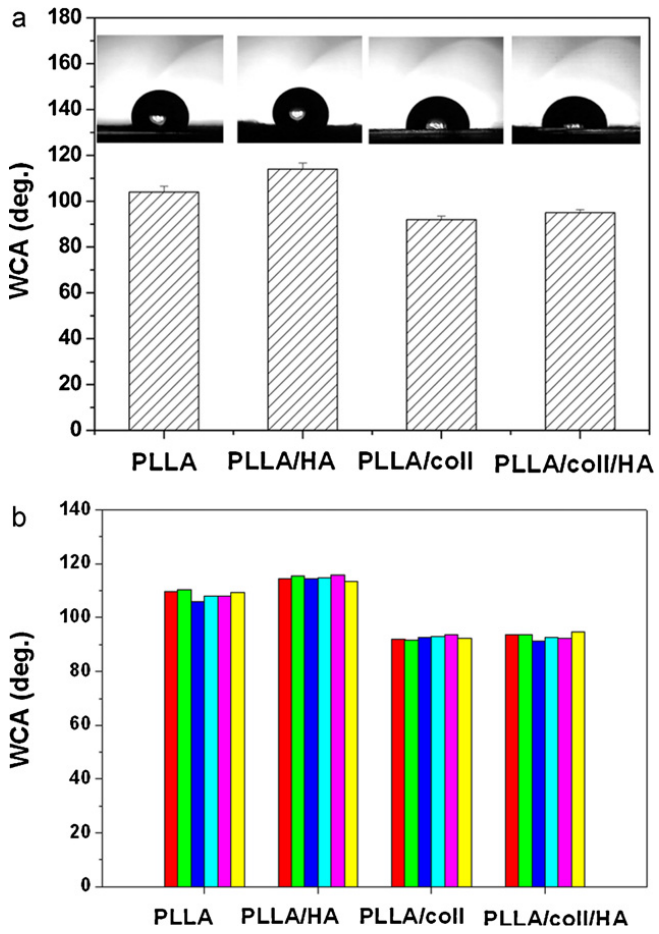
- the exfoliated MMT/PLLA solution was electrospun to provide MMT-reinforced PLLA nanofibers], which were subsequently mixed with NH4HCO3/NaCl salt particles and mechanically entangled by a cold compression-molding process in a solid state. After leaching out the salts, the resultant solid scaffolds exhibited a robust porous structure containing a dual-porosity network in the ranges ofa few nanometers and a few hundred micrometers., Biomaterials



(b)

*DUAL POROUS SCAFFOLDS PREPARED BY ELECTROSPINNING

Water Contact angle

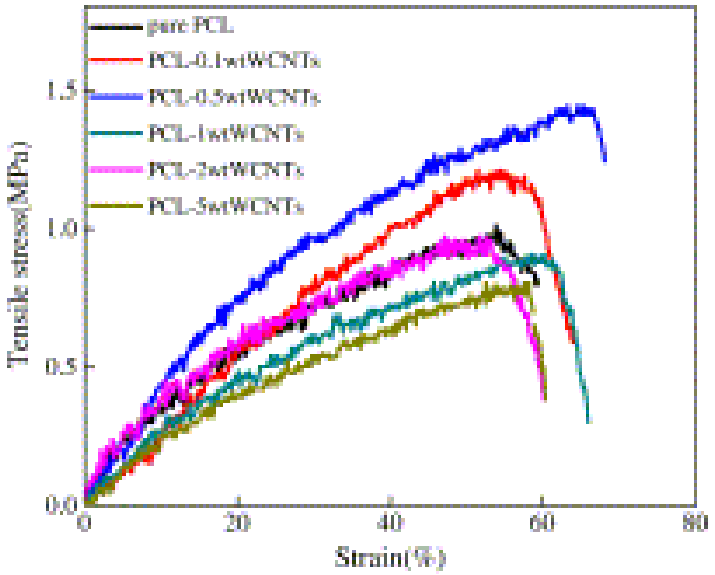


The attachment and the growth of cells need materials with a hydrophilic surface. The hydrophilicity of a scaffold can be determined by measuring the WCA

Water contact angle (WCA) of electrospun scaffolds: (a) averaged WCAs; (b) WCAs measurement changed with the time: measured at 2 s, 4 s, 6 s, 8 s, 10 s and 12 s. Bars correspond to the mean±standard deviation for n≥10 measurements

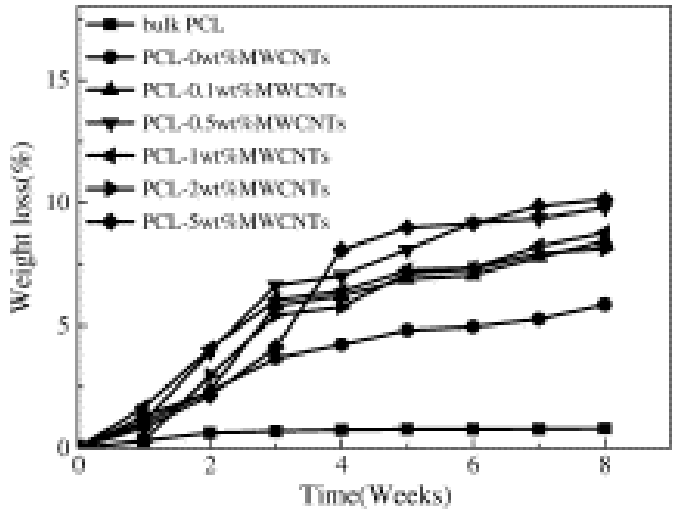
D. Cao et al,Colloids and Surfaces B: Biointerfaces, *2011*

Tensile properties



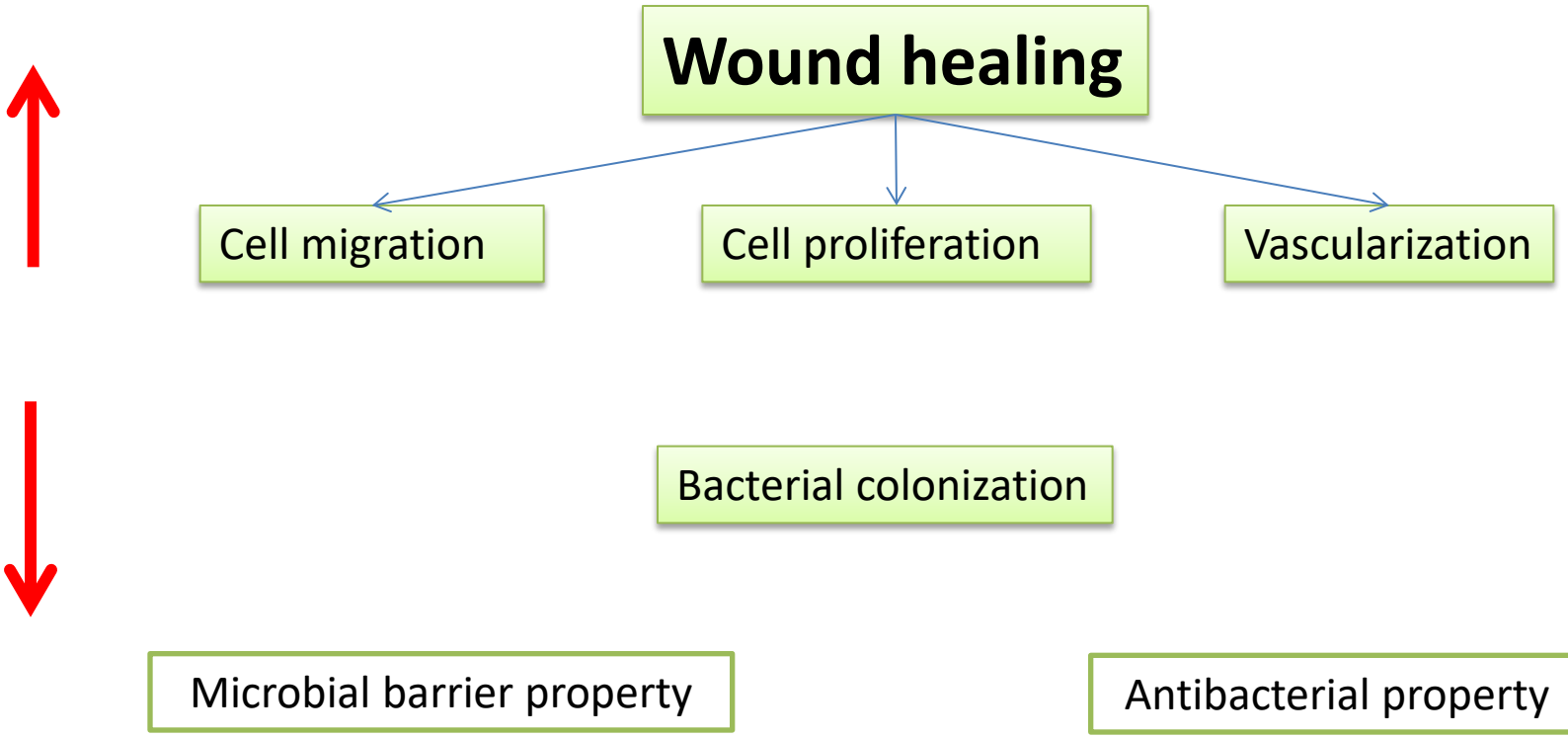
Typical tensile stress–strain curves of pure PCL and PCL–MWCNTs nanofiber membranes

In vitro degradation

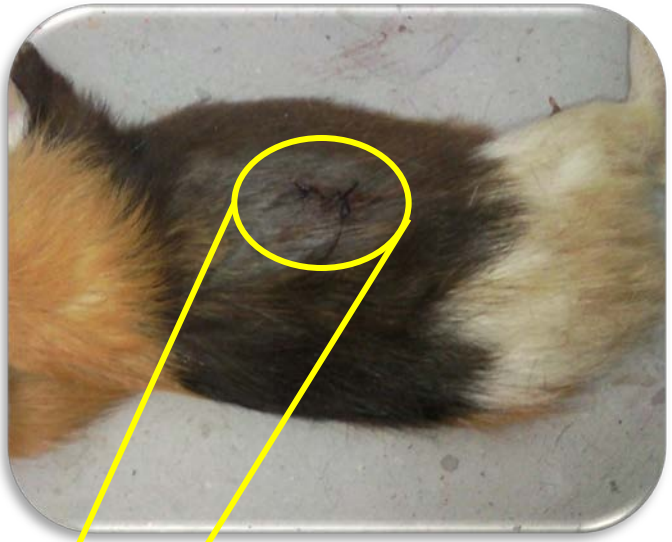


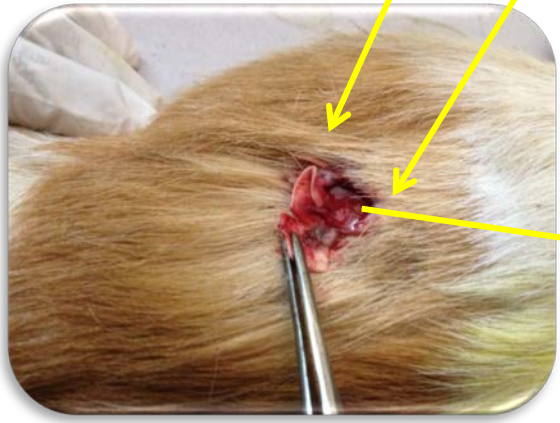
In vitro degradation of pure bulk PCL and electrospun PCL–MWCNTs nanofiber membrane in PBS (pH 7.4) at 37 °C.

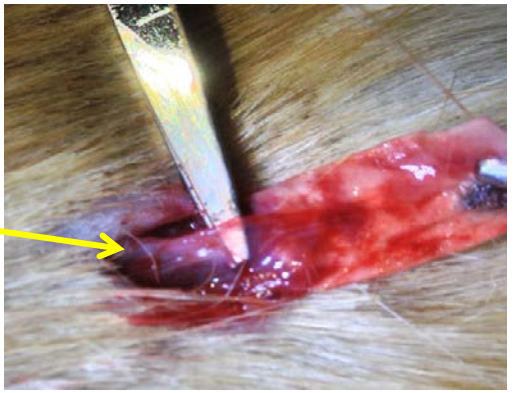
Wound healing



Implantation in Guinea pigs







Wound Healing Activity

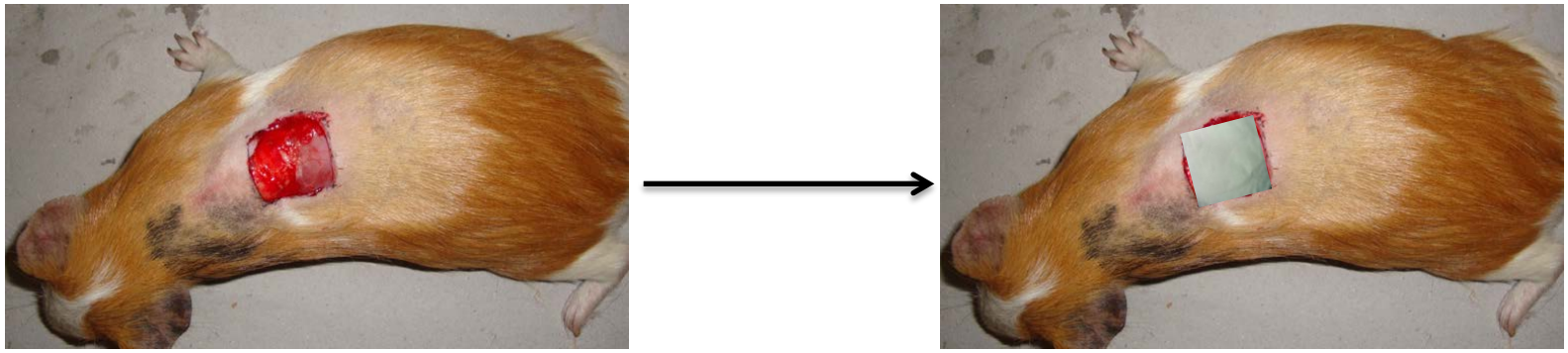
A 2 X 2 cm full thickness skin excision wound was made and PCL membranes with 2 X 2 cm dimensions were sutured on the wounds and the photographs were taken each day until the wounds were perfectly healed.

Positive control (Povidone- Iodine (Betadine® ointment) and negative control groups were also maintained.

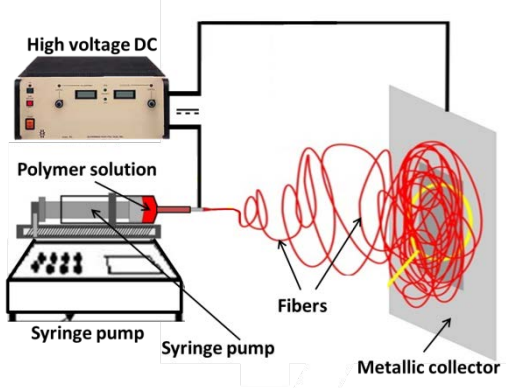
The percentage of wound healing was calculated using the formula,

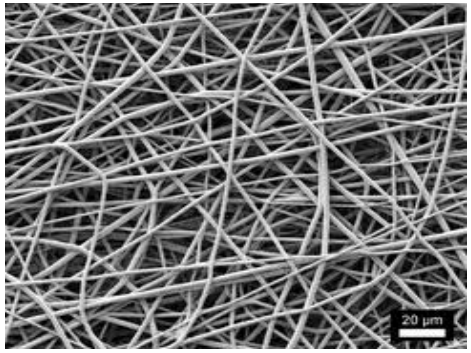
$$W^{\%} = \frac{[WA^0 - WA^t]}{WA^0} \times 100$$

where $W^{\%}$ is the percentage of wound healing, WA^0 is the area of wound at 0^{th} day and WA^t is the area of wound after different days of healing.

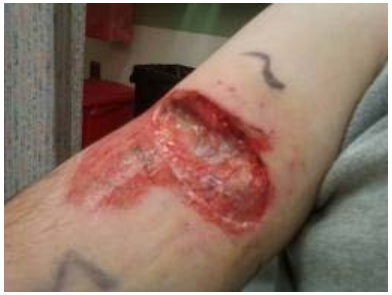


Present approach

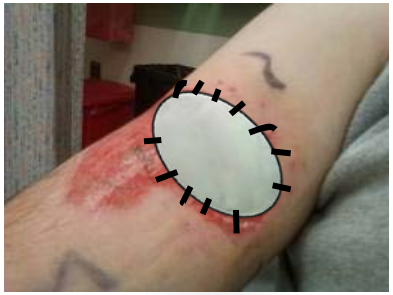




Wound

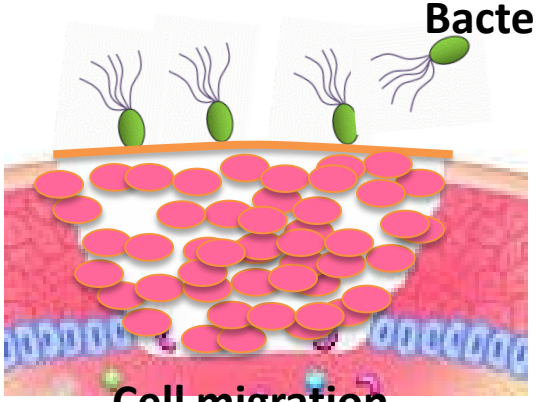


Skin substitute application



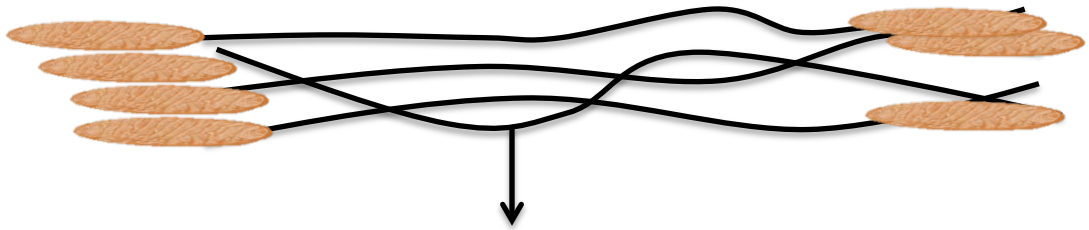
Healed wound

Bacteria



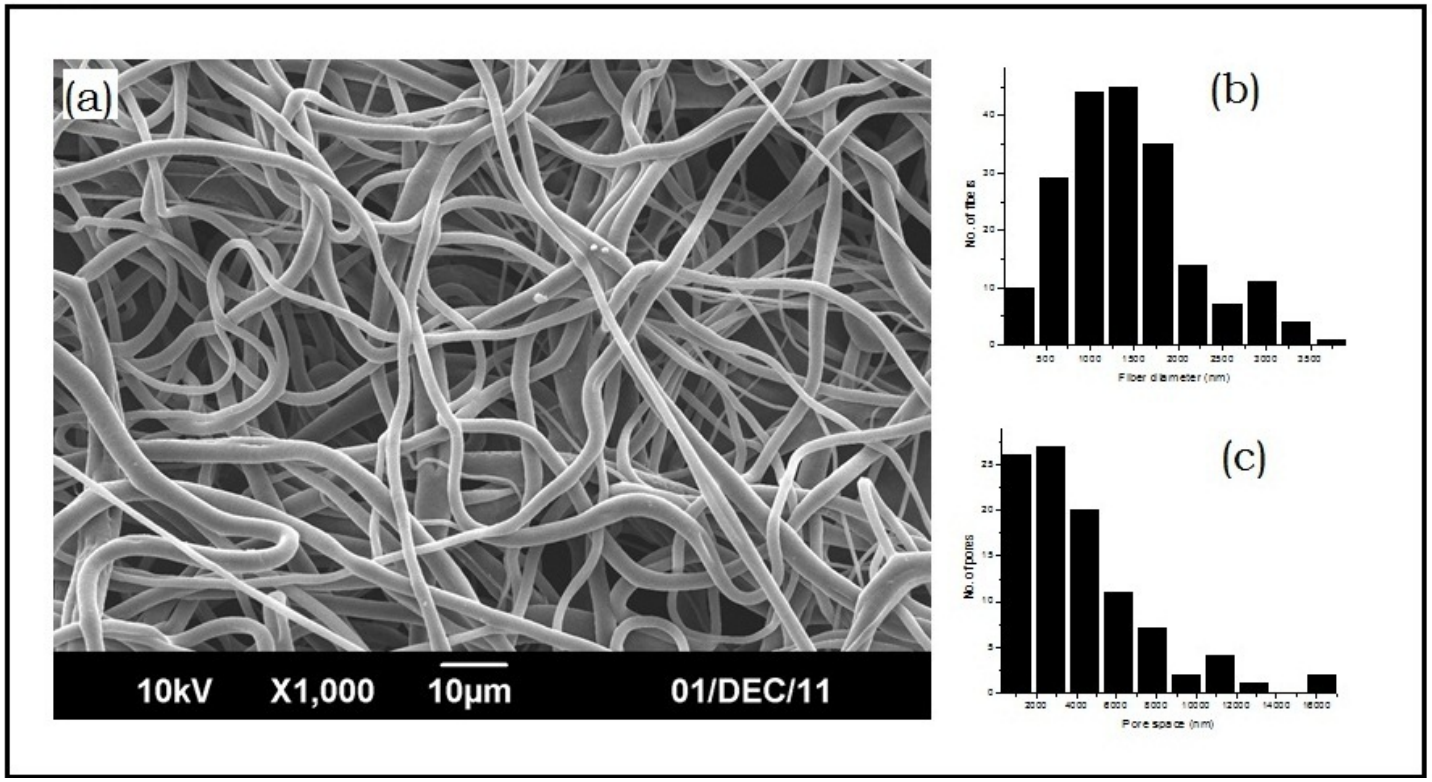
Cell migration

Cell migration through electrospun membranes



Electrospun fibres

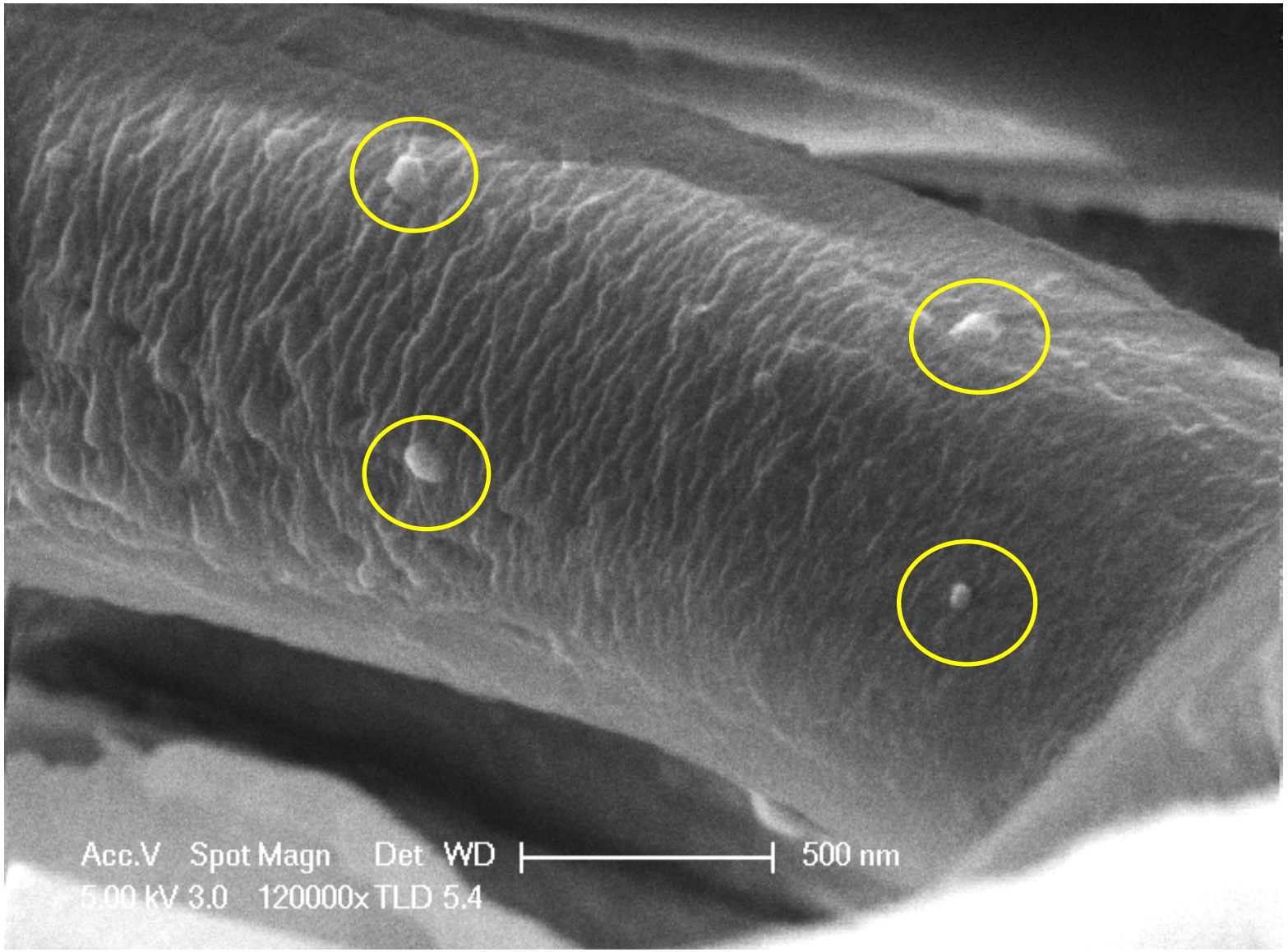
Morphology



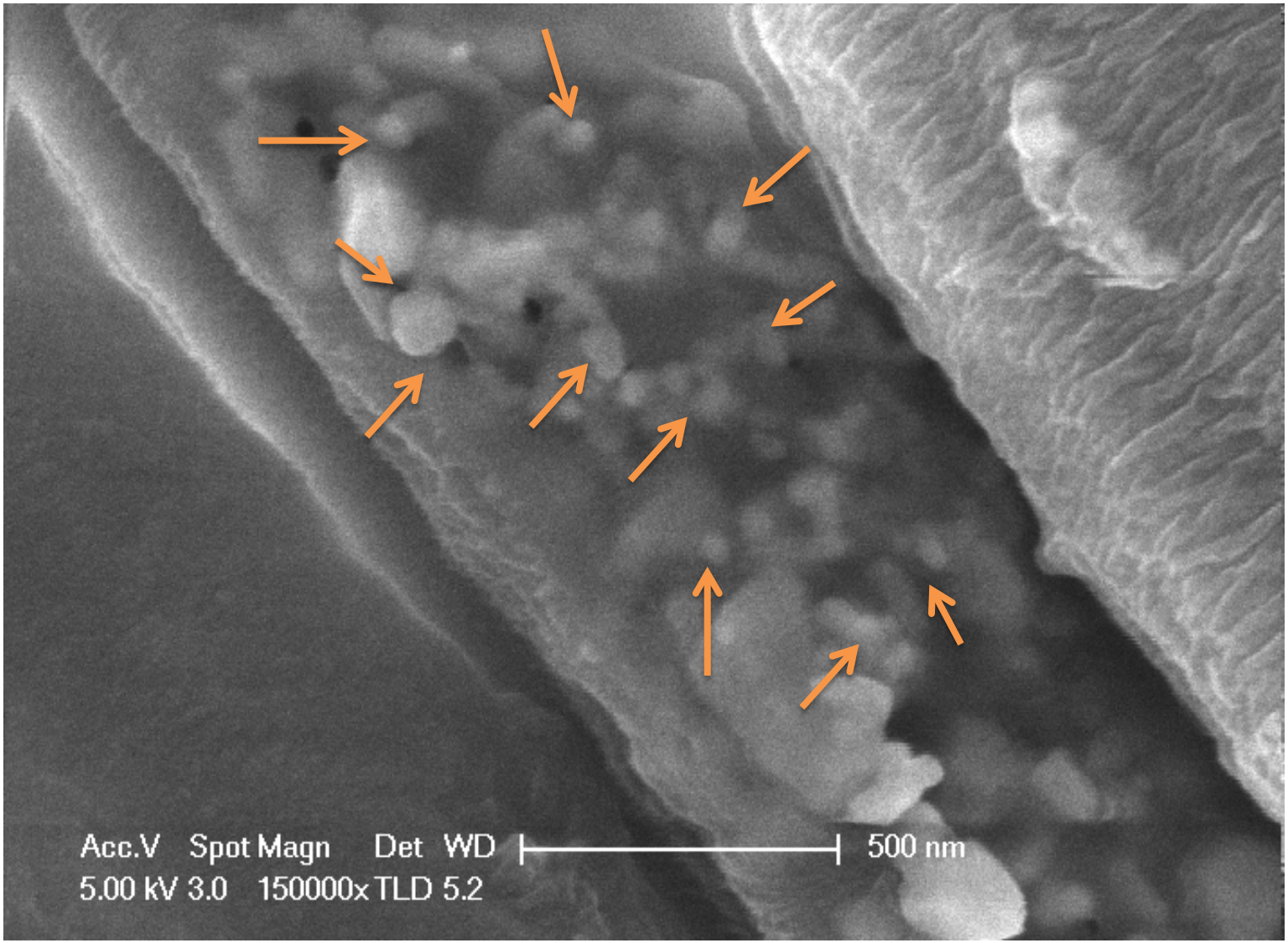
Scanning Electron Micrograph of electrospun neat polycaprolactone membrane (a), fiber diameter distribution and the pore space distribution (c).

Augustine, R., Malik, H. N., Singhal, D. K., Mukherjee, A., Malakar, D., Kalarikkal, N., & Thomas, S. (2014). *Journal of Polymer Research*, *21*(3), 1-17.

Morphology, RSC advances, 2015



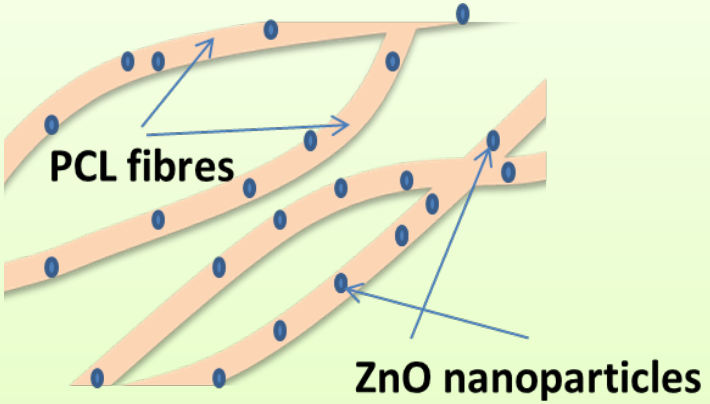
Morphology, RSC Advances, 2015

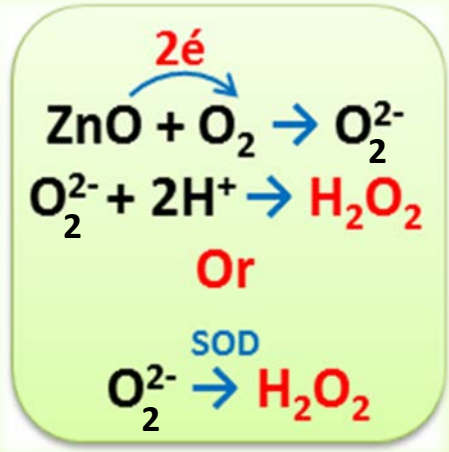


The approach

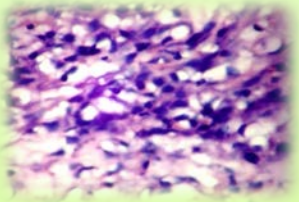
Electrospun PCL/ZnO nanoparticles membrane

NANOSCALE,2015

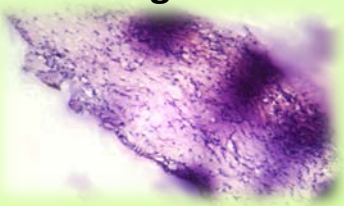




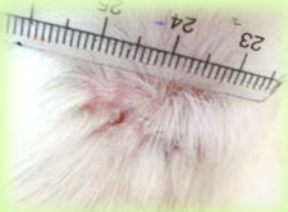
Cell proliferation



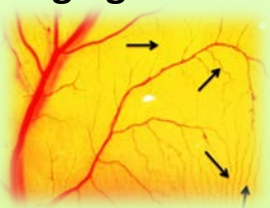
Cell migration



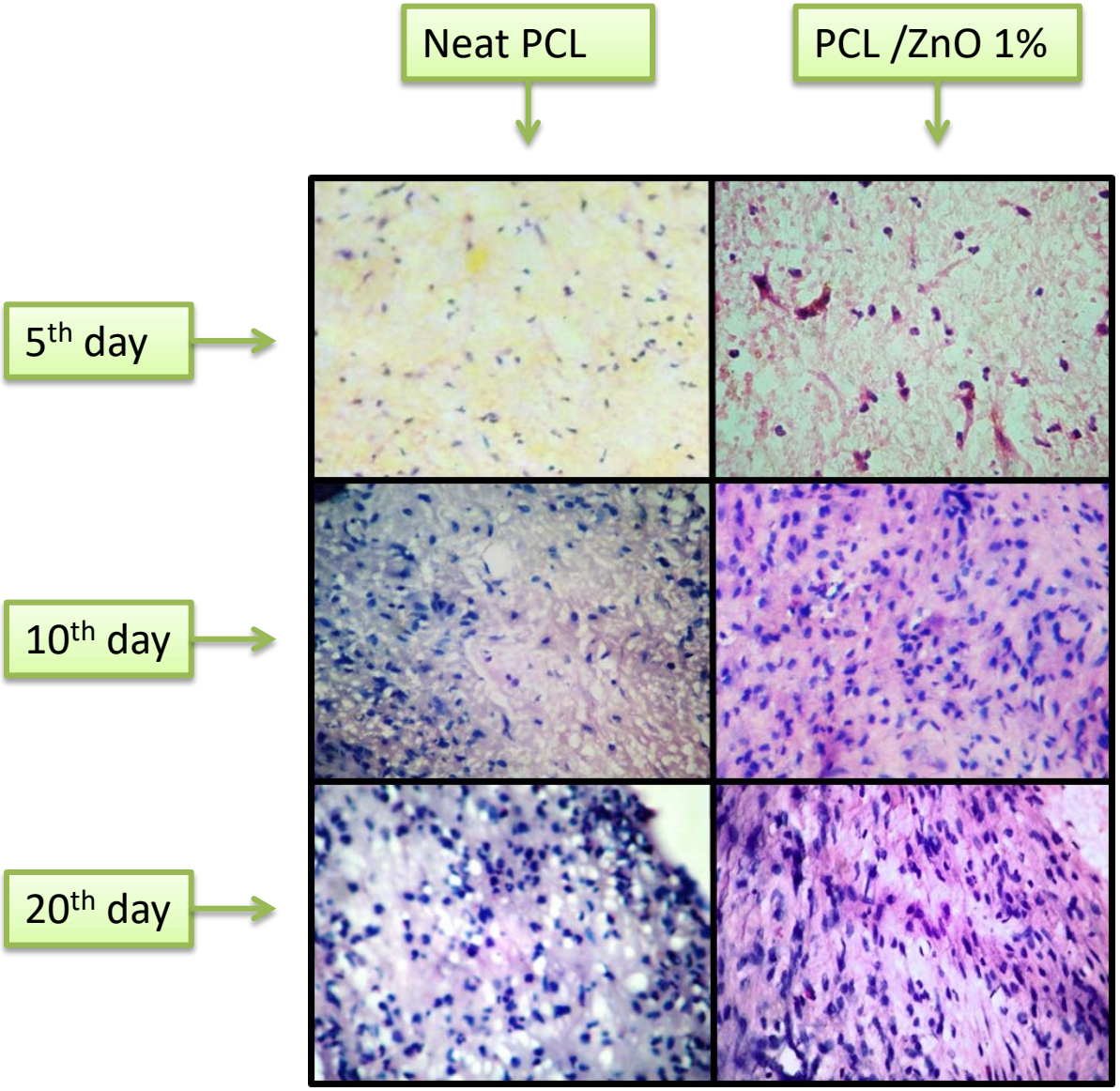
Wound healing



Angiogenesis

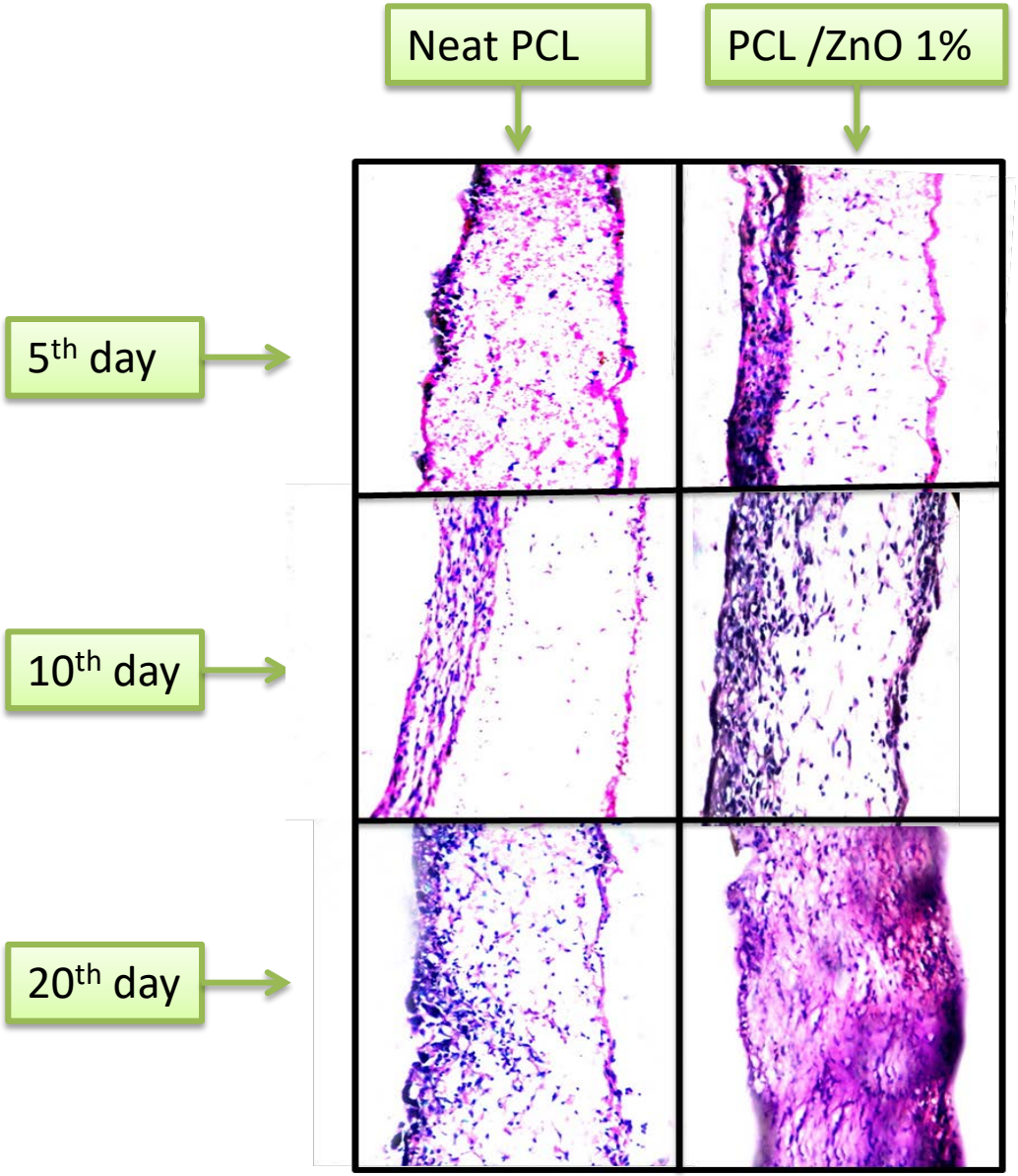


Histology



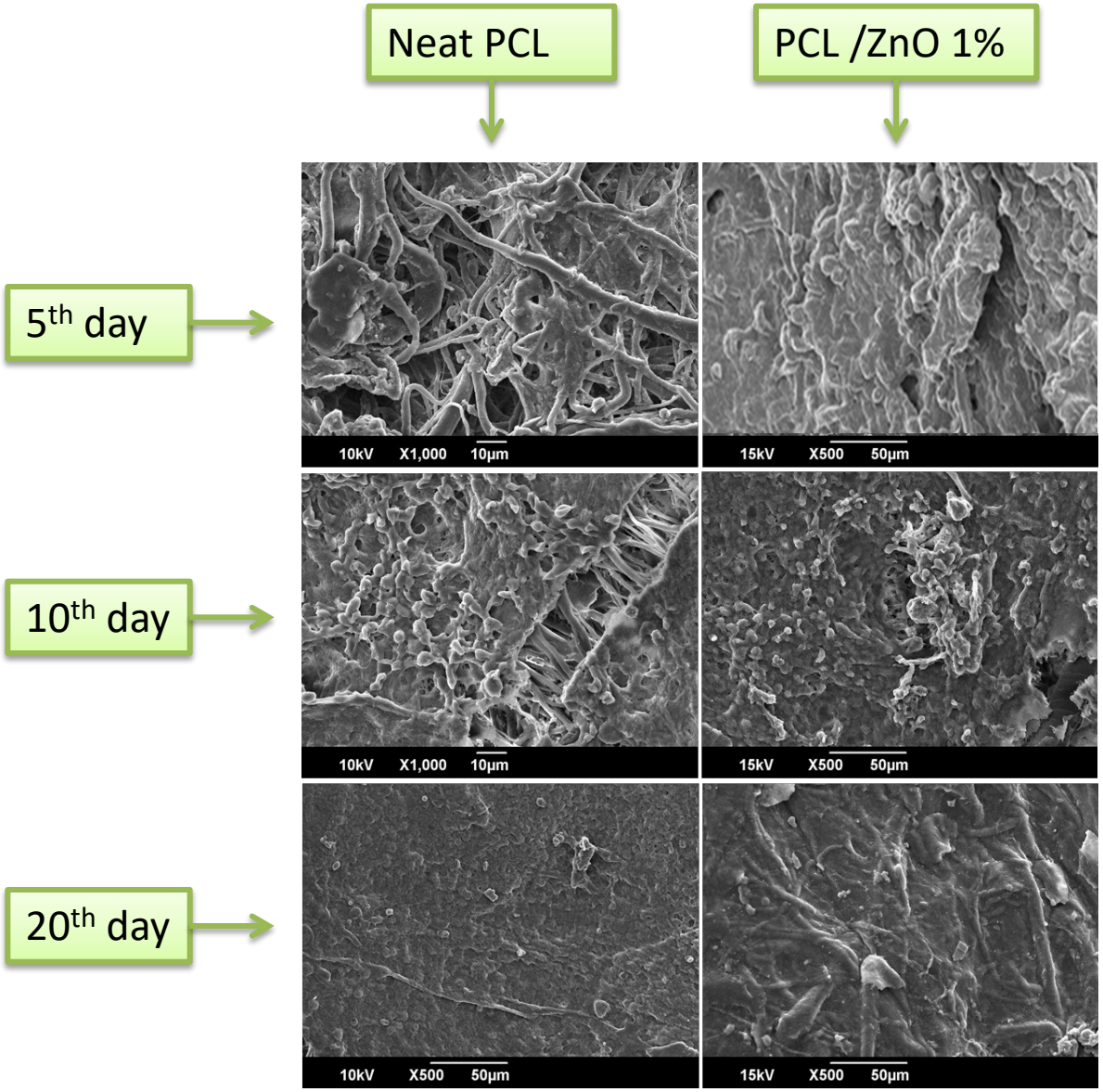
Augustine, R., Dominic, E. A., Reju, I., Kaimal, B., Kalarikkal, N., & Thomas, S. (2014). ***RSC Advances*** 4 no. 48 (2014): 24777–24785.

Histology



Augustine, R., Dominic, E. A., Reju, I., Kaimal, B., Kalarikkal, N., & Thomas, S. (2014). ***RSC Advances*** 4 no. 48 (2014): 24777–24785.

SEM after implantation



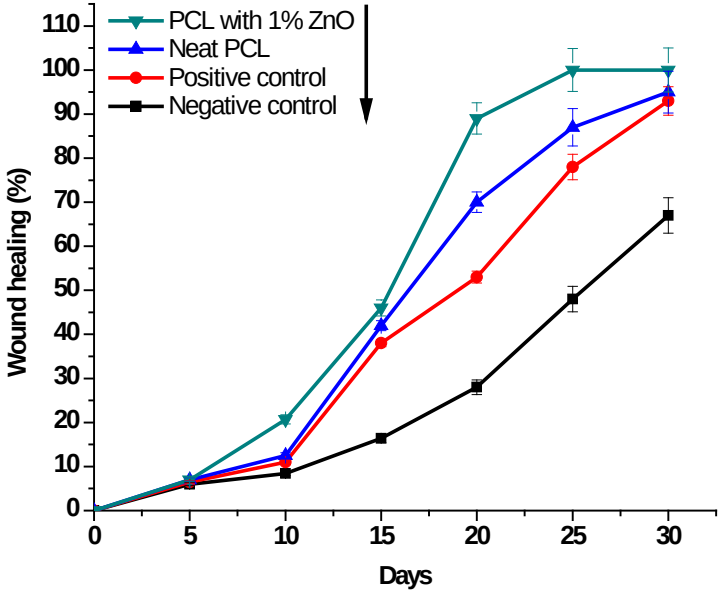
Augustine, R., Dominic, E. A., Reju, I., Kaimal, B., Kalarikkal, N., & Thomas, S. (2014). ***RSC Advances*** 4 no. 48 (2014): 24777–24785.

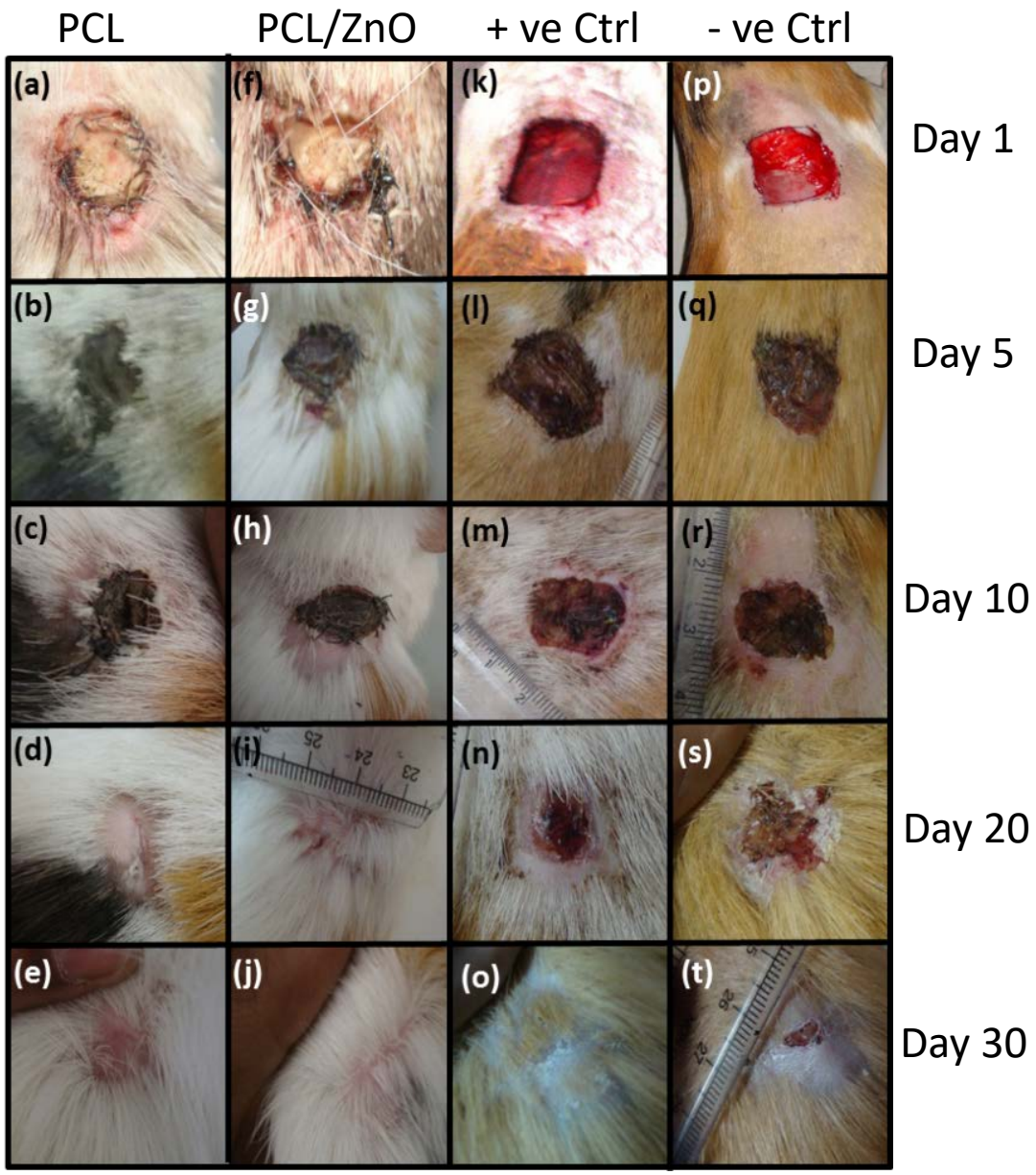
Wound healing

Percentage of wound healing was calculated using the formula,

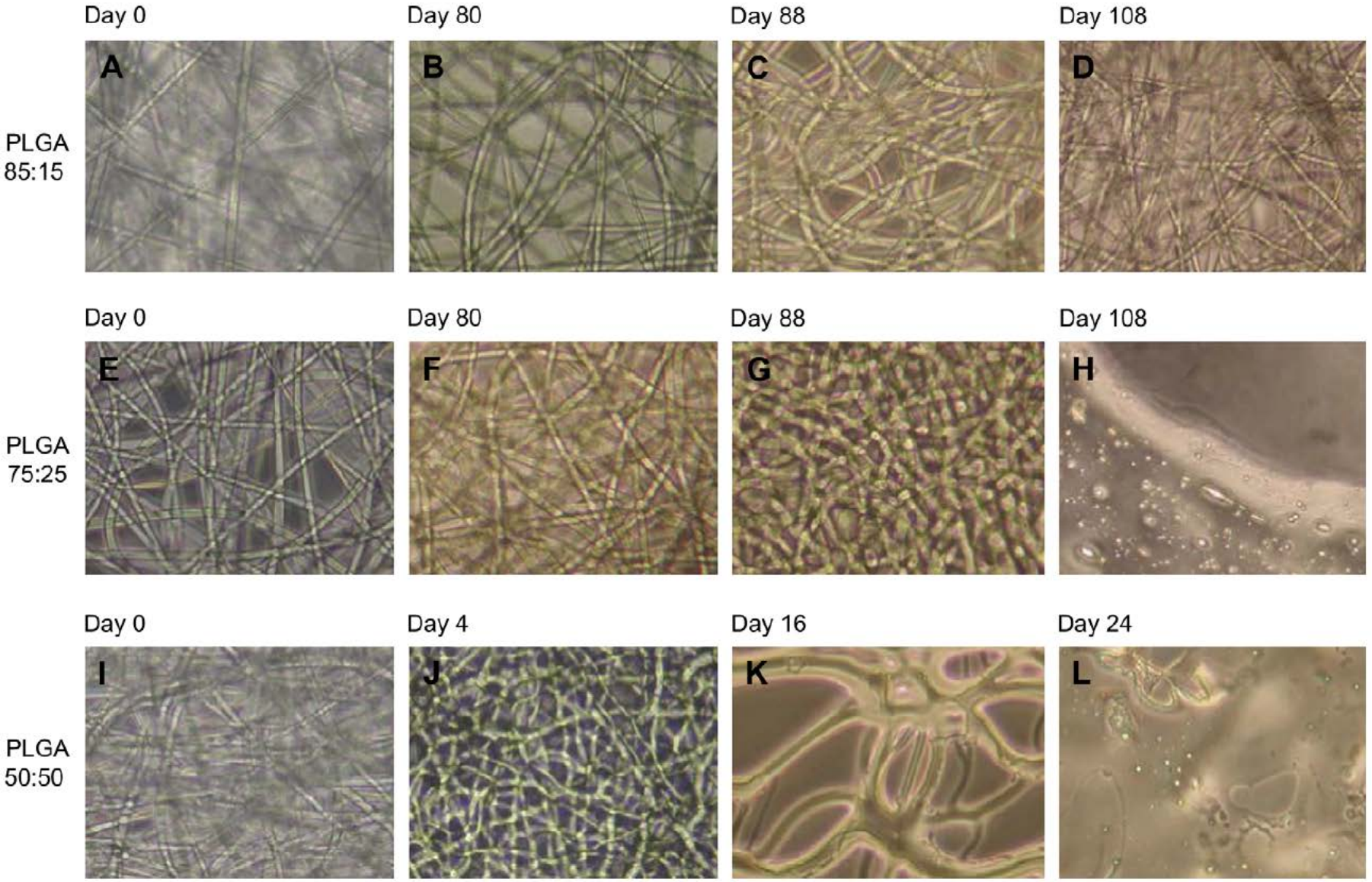
$$W^{\%} = \frac{[WA^0 - WA^t]}{WA^0} \times 100$$

Where $W^{\%}$ is the percentage of wound healing, WA^0 is the area of wound at 0^{th} day and WA^t is the area of wound after different days of healing.

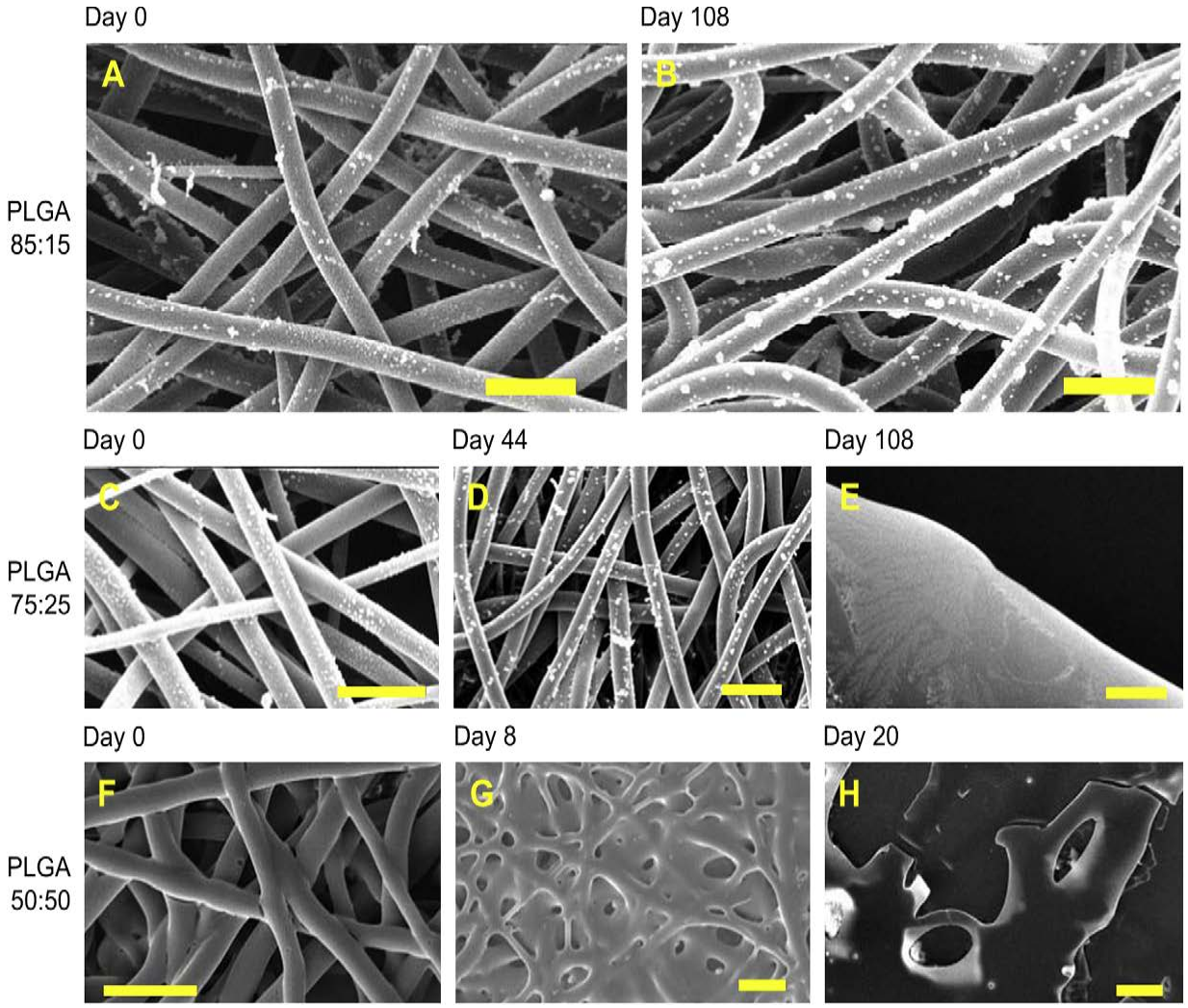




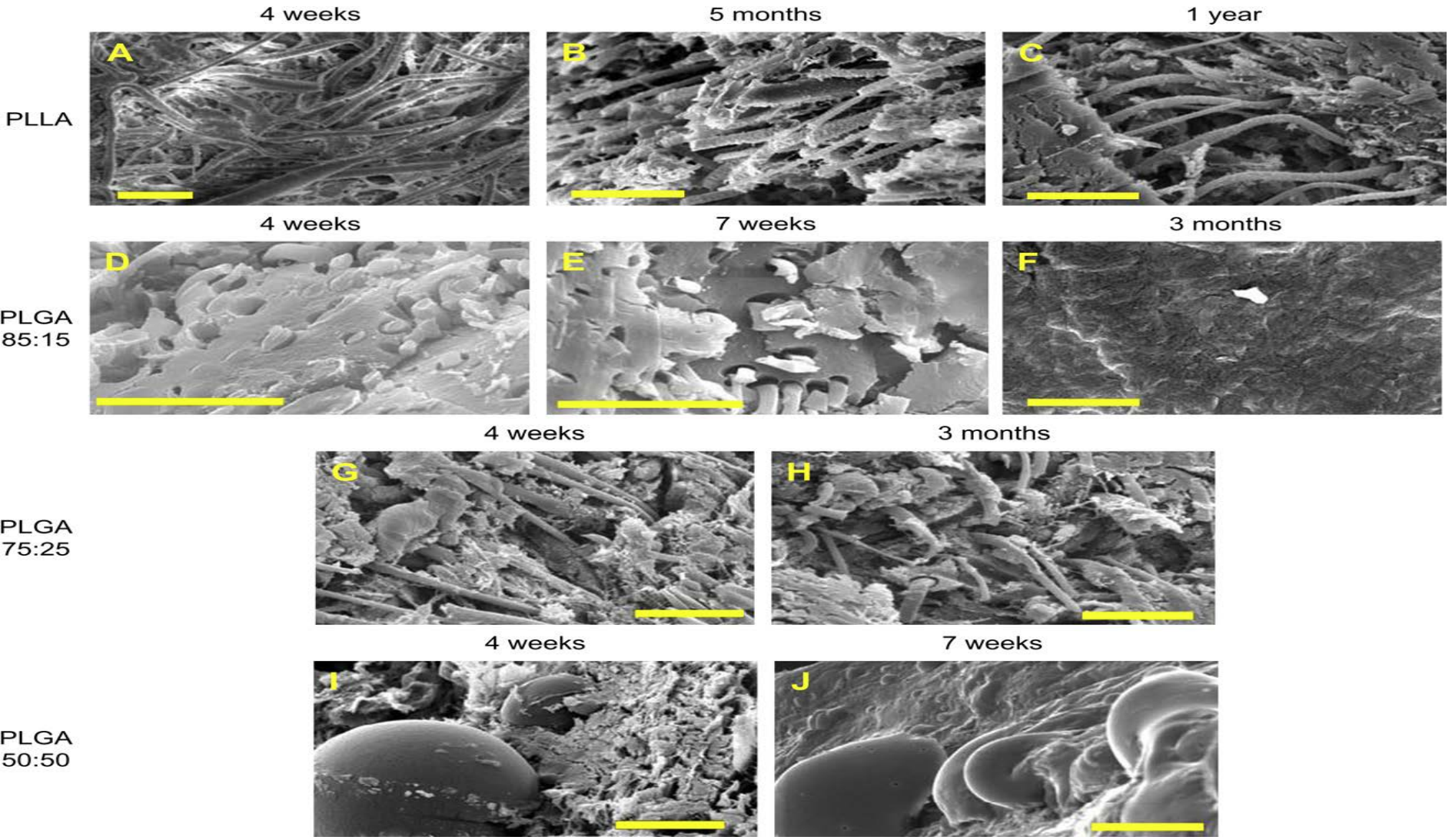
Augustine, R., Dominic, E. A., Reju, I., Kaimal, B., Kalarikkal, N., & Thomas, S. (2014). ***RSC Advances*** 4 no. 48 (2014): 24777–24785.



Light microscope images of PLGA 85:15 (A–D), 75:25 (E–H), and 50:50 (I–L) electrospun polymers after immersion in Ringers solution at 37^0 C in 5% CO_2 for various lengths of time as indicated in the figure. As can be seen as the percentage of PGA increased the polymer fibres lost integrity faster.



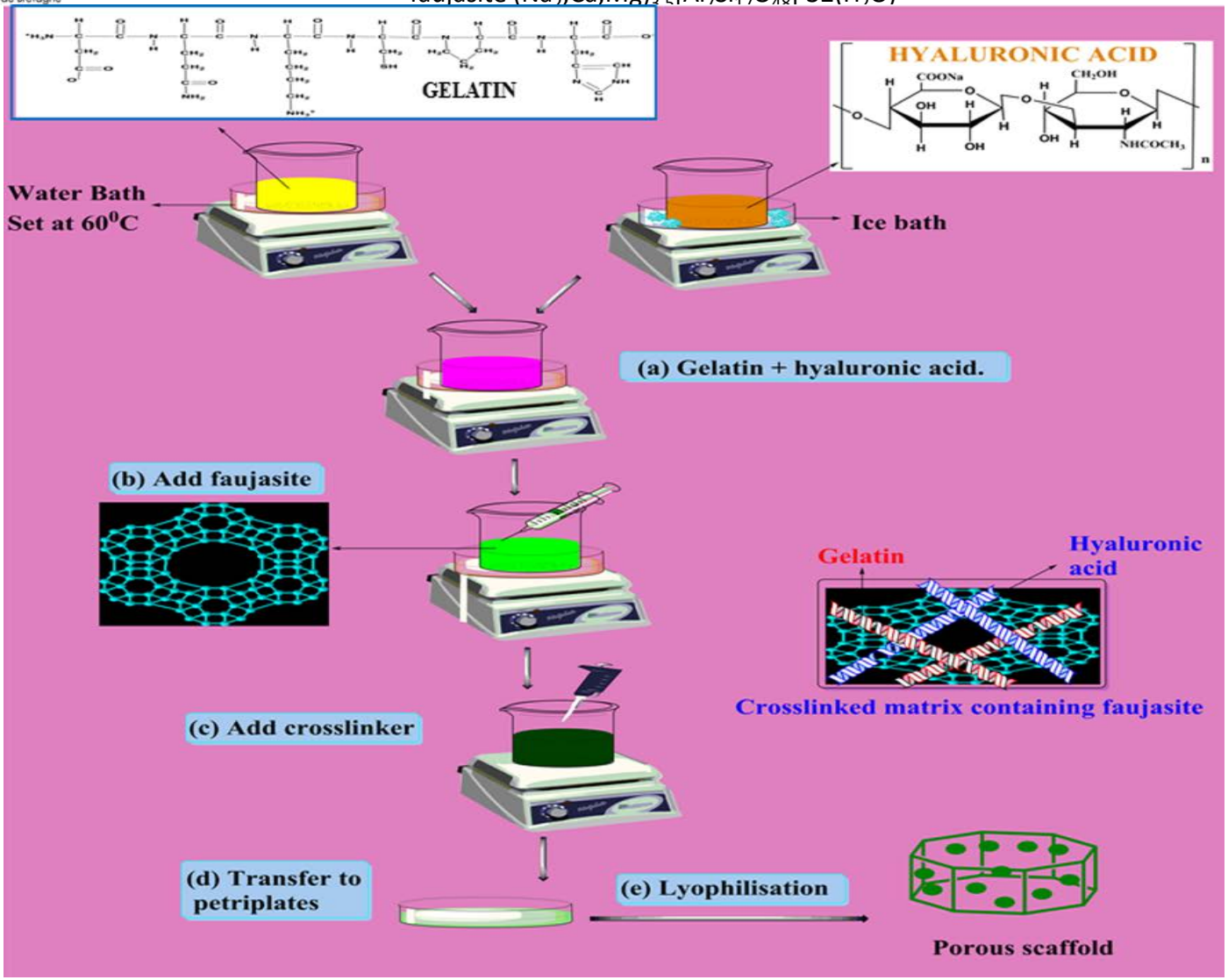
SEM micrographs of electrospun PLGA 85:15, (A þ B), 75:25 (C–E), and 50:50 (F–H) after being immersed in Ringers solution at 37 C in 5% CO2 for various lengths of time as indicated in the figure. Scale bar ¼ 10 mm.



SEM micrographs of PLLA (A–C), PLGA 85:15 (D–F), 75:25 (G–H), 50:50 (I–J) following implantation into the flank of adult male Wistar rats at the time points indicated(4 weeks to 1 year).

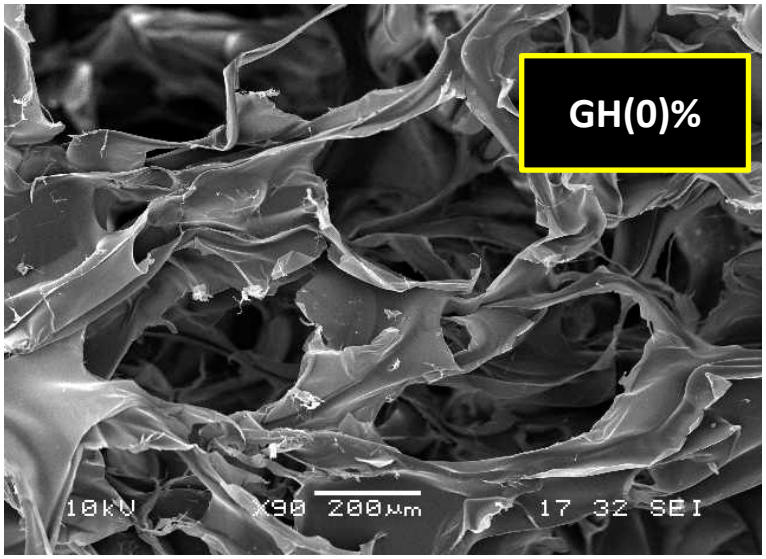
METHOD, ACS APPLIED MATERIALS AND INTERFACES, 2014,

faujasite $(Na_2,Ca,Mg)_{3.5}[Al_7Si_{17}O_{48}]\cdot 32(H_2O)$

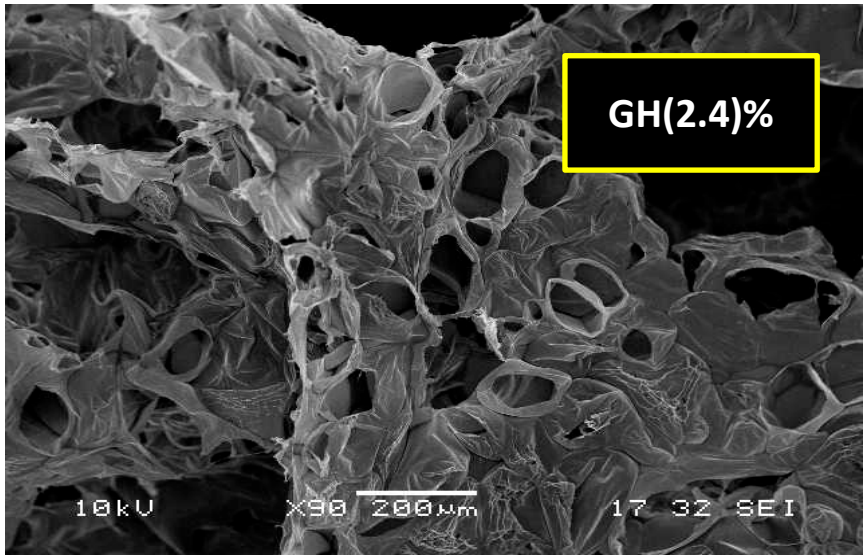


:PHOLOGY ANALYSIS, ACS APPLIED MATERIALS AND INTERFACES, 2014 (THOMAS)

50-2000µm

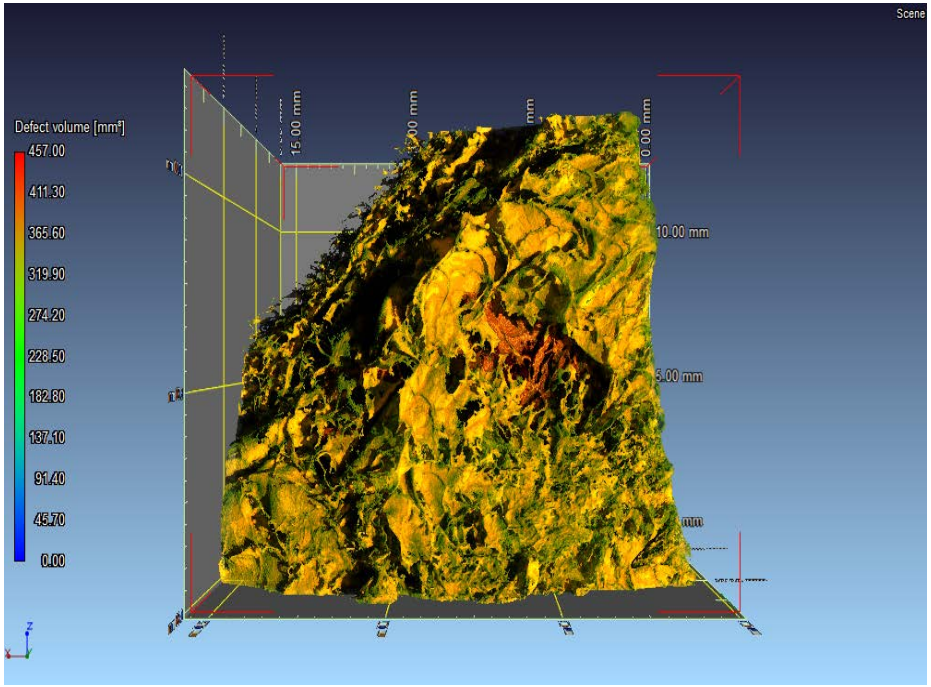


10-250µm



POROSITY ESTIMATION

10-350 µm, tomography



Porosity- 90.6%

GH(2.4%), ACS APPLIED MATERIALS AND INTERFACES, 2014

SURFACE PROPERTIES

SAMPLES	Contact angle values (θ)
GH(0%)	92±2
GH(0.24%)	97±1
GH(0.48%)	108±2
GH(2.4%)	117±3
GH(4.8%)	77±2

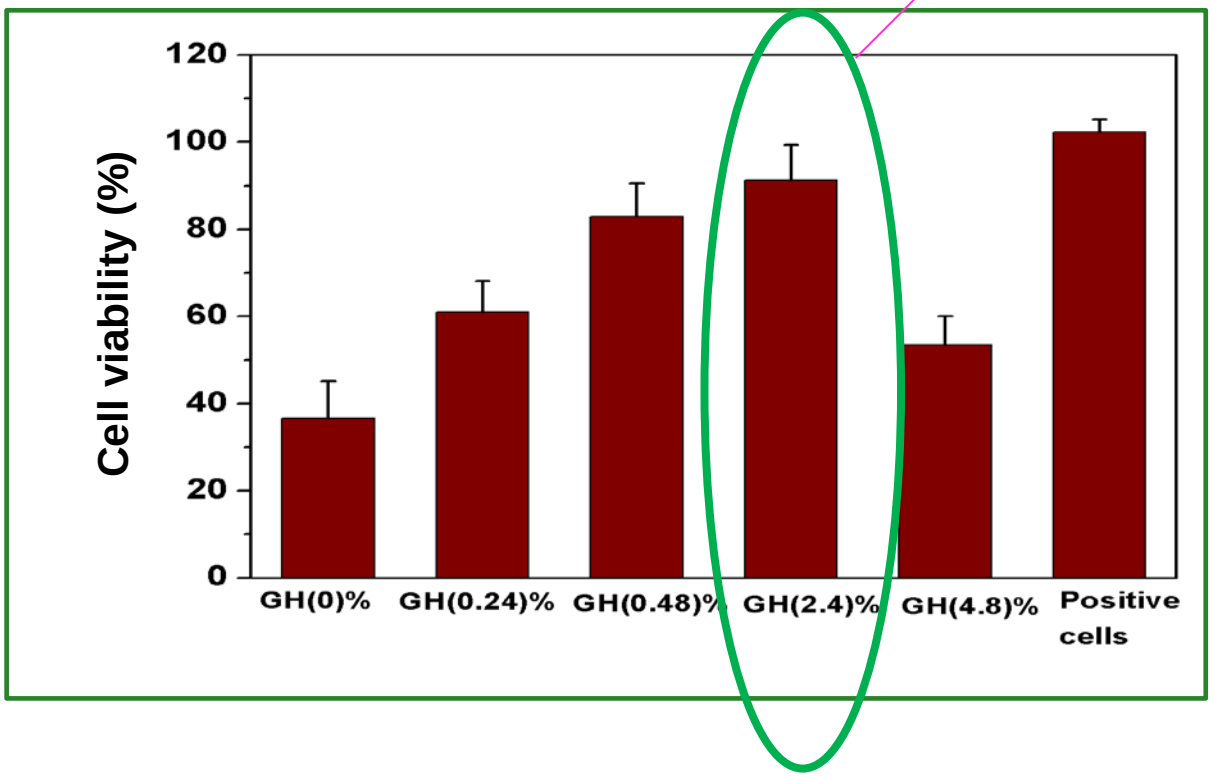
HIGHLY HYDROPHILIC-

MODERATELY HYDROPHOBIC-

Cell Viability

92%

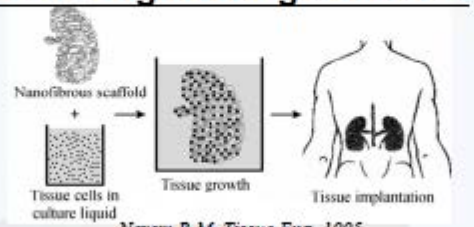
Highest among 3 scaffolds



Conclusion

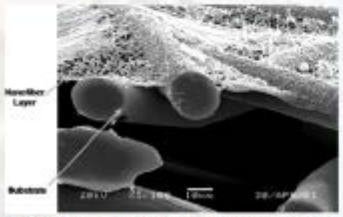
Application ares of Electrospun Nanofibers

Tissue engineering scaffolds



Nerem R M, Tissue Eng, 1995

Filtration

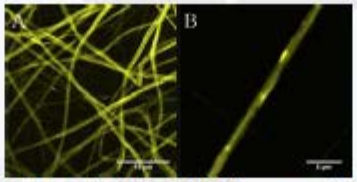


K. Graham, Adv. Filtr. Sep. Technol., 2002

Wound healing

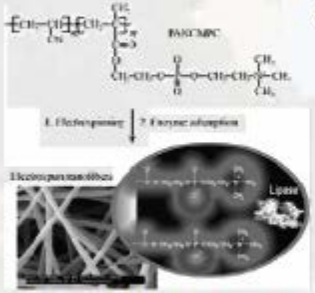
Khil M-s, J Biomed Mater Res Part B: Appl. Biomater, 2003

Release control



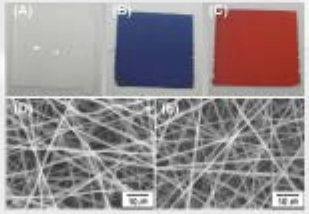
T.G. Kim et al. / International Journal of Pharmaceutics, 338, 276, 2007

Catalyst and enzyme carriers



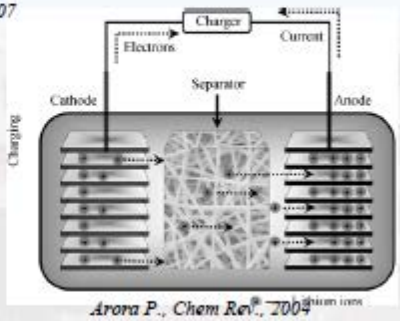
Huang X-J, Rapid Commun, 2006

Sensors



Jong-Man Kim, Macromol. Chem. Phys. 2008

Energy storage



Arora P., Chem Rev., 2004

Acknowledgements

Co-workers: Neethu Ninan, Robin Augustine and Eldhow Elias

Government Funding

DST, Nanomission (Prof. CNR Rao)

CSIR, DBT, ICMR, UGC, DRDO, DIT, TWAS,BRNS

Industrial Funding

Surface treat, Czech

Collaborators

- Prof. Yves Grohens, Uni of South Brittany, France
- T W Wong, Uni Tech Mara, Malaysia
- Pushpagiri School of Medical Sciences, India

Thank you for Your Attention

Nano Group


LOCATION OF KERALA
IN INDIA
N
JAMMU & KASHMIR
CHINA
PAKISTAN
UTTARANCHAL
DELHI
NEPAL
BHUTAN
Patna
JHARKHAND
Calcutta
CHHATTISGARH
Mumbai
(Bombay)
ARABIAN SEA
BAY OF BENGAL
KARNATAKA
KERALA
Thiruvananthapuram
Map not to Scale
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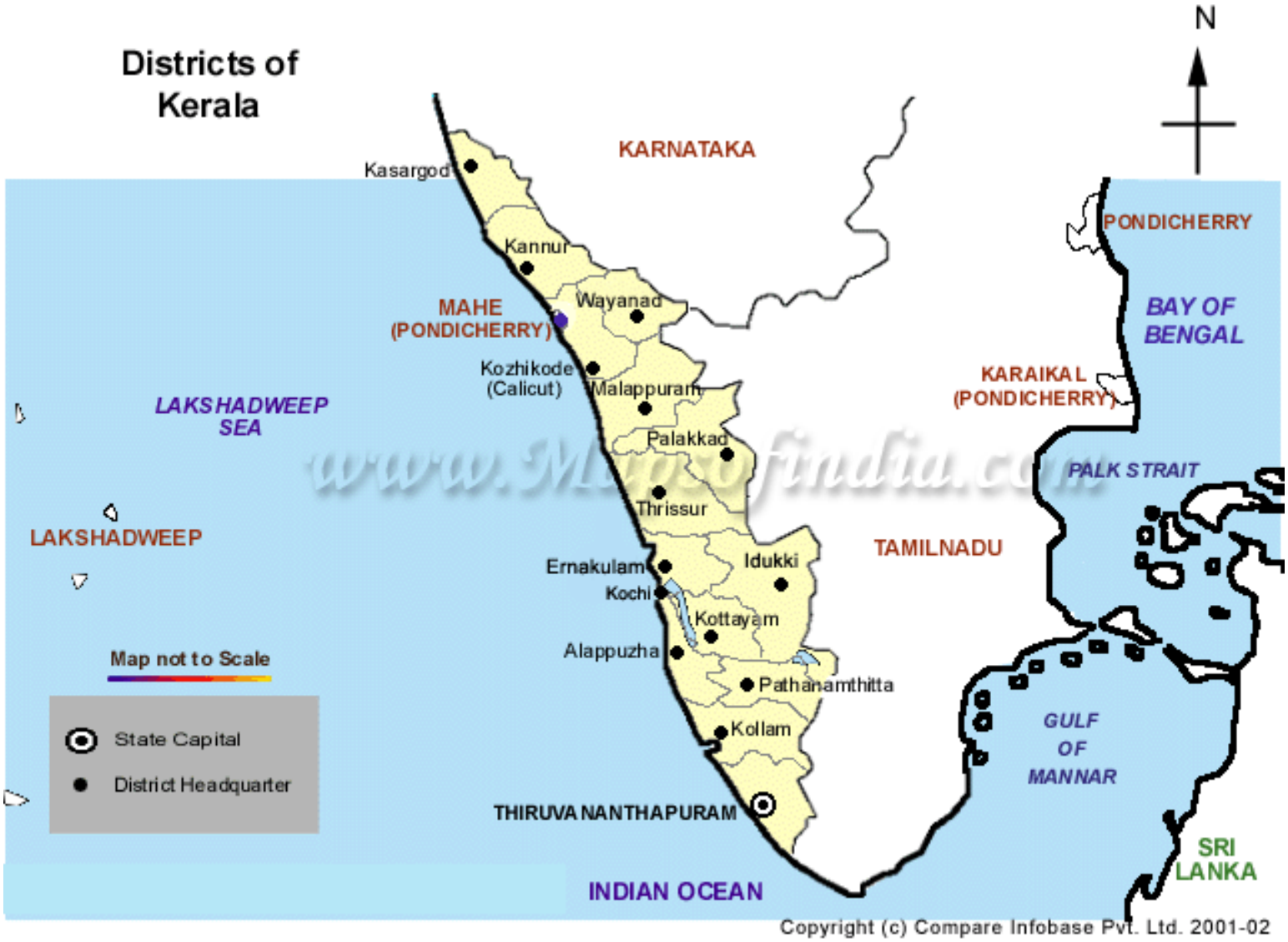
Districts of Kerala
N
KARNATAKA
Kasargod
Kannur
Wayanad
MAHE (PONDICHERRY)
Kozhikode (Calicut)
Malappuram
Palakkad
Thrissur
Ernakulam
Idukki
Kochi
Kottayam
Alappuzha
Pathanamthitta
Kollam
THIRUVANANTHAPURAM
PONDICHERRY
BAY OF BENGAL
KARAIKAL (PONDICHERRY)
PALK STRAIT
TAMILNADU
LAKSHADWEEP SEA
LAKSHADWEEP
GULF OF MANNAR
SRI LANKA
INDIAN OCEAN
Map not to Scale
State Capital
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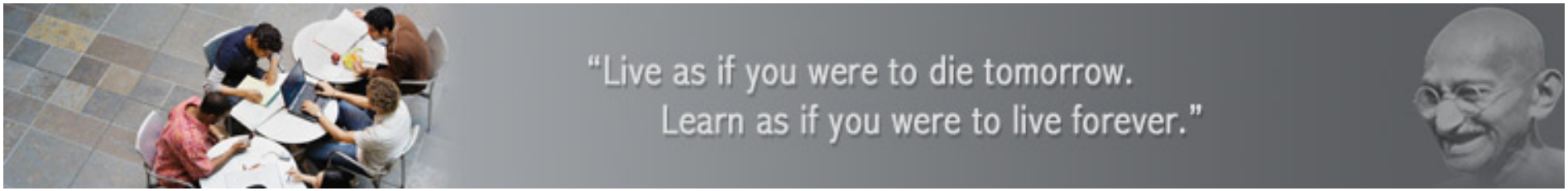

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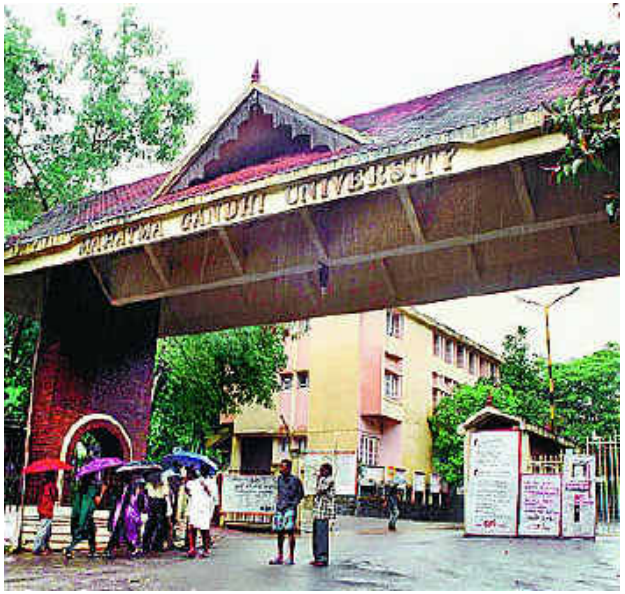
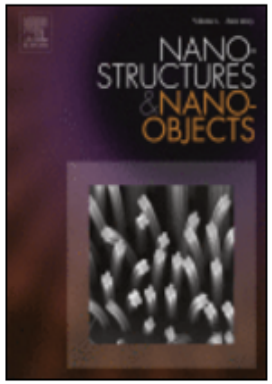


"The aim of University education should be to turn out true servants of the people, who would live and die for the country's freedom" – Mahatma Gandhi



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Influence of the crystal structure on the physical properties of monoclinic ZrO_2 nanocrystals
Pages 1-6
Minori Taguchi, Akiyuki Matsushita, Tetsuo Uchikoshi, Yoshio Sakka, Seiichi Takami, Toshitaka Funazukuri, Takashi Naka
Abstract | Close graphical abstract


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