Biohybrid Electrospun Nanofibers:
Encapsulation of Cells into Electrospun Fibers

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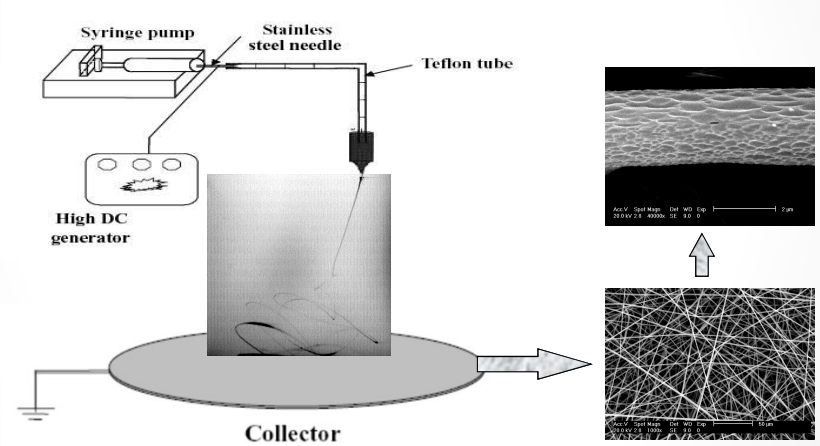
PRESENTATION OUTLINES

- Background; Electrospinning Technique.
- Encapsulation of Cells into Electrospun Nanofibers
  - Introduction
  - Examples

Outlook.com team, 12/18/2017 **Ot1**

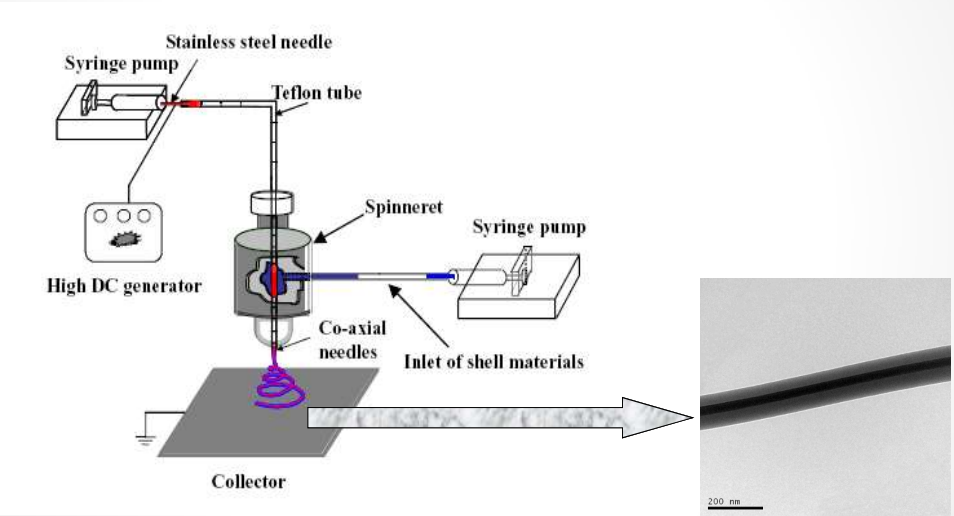
Background; Electrospinning Technique

- Electrospinning is a common method to produce nanofibers with a diameter in the range of 100 nm or even less.



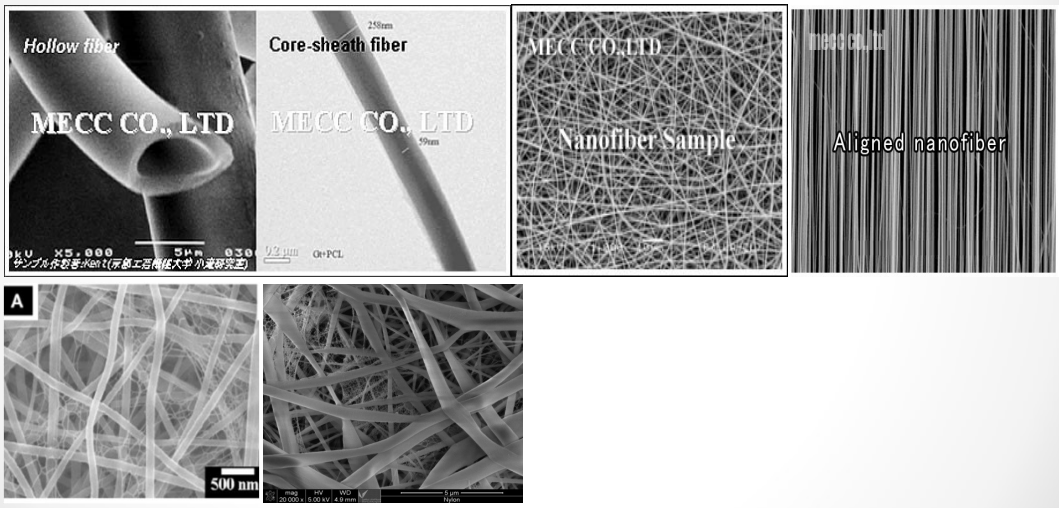
Background; Electrospinning Technique

- Coaxial Electrospinning



Background; Electrospinning Technique

- Electrospun Nanofibers Architectures



Background; Electrospinning Technique

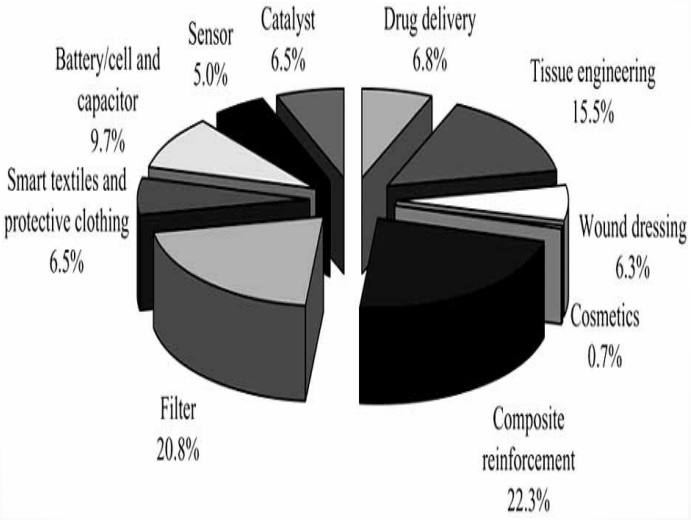
- Electrospinning offers advantages like
  - Simplicity.
  - Low cost.
  - High efficiency.
  - High yield.
- Properties of electrospun nanofibers
  - Electrospun nanofibers are micro-nano (40-2000 nm) in nature.
  - high aspect ratio (*length to width ratio*),
  - high porosity
  - large surface area

Background; Electrospinning Technique

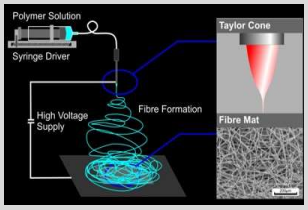
- Welcome To World Nanofibers

Background; Electrospinning Technique

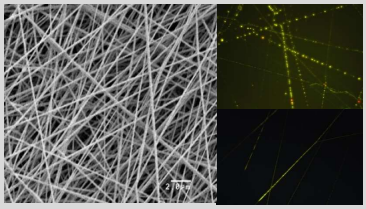
- Applications of Nanofibers



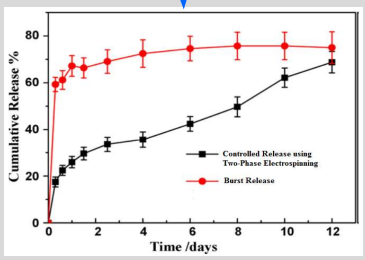
Biomedical Applications



Electrospinning



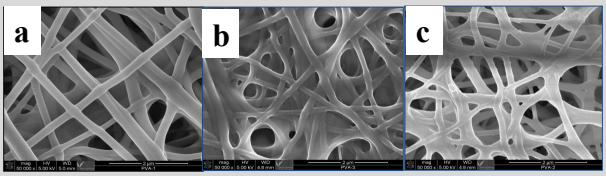
Electrospun nanofibers encapsulated with drug



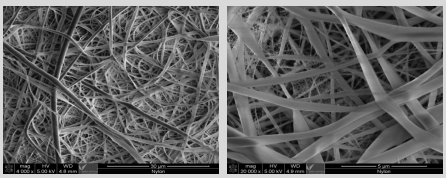
Applications

Wound dressing & healing

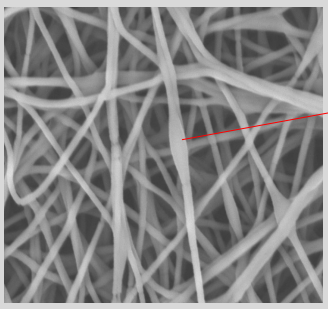
Nanofibers made drug delivery



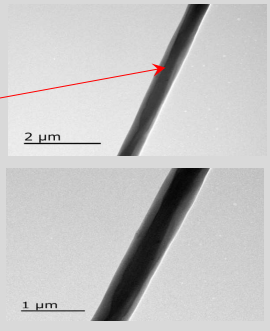
SEM images of electrospun nanofibers containing MTZ; (a) electrospun mat; (b) electrospun mat-alc; (c) electrospun mat-h.



SEM images of electrospun nylon-6 nanofiber containing.



TEM images of Silk/PEO nanofibers with dexamethasone



Background; Electrospinning Technique

- Biomedical Applications; Drug Delivery
  - Electrospun nanofibers have exhibited many advantages;
    - The drug loading is very easy to implement via electrospinning process (*More than one drug can be encapsulated and the high applied voltage used in the electrospinning process had little influence on the drug activity*).
    - The high specific surface area
    - Short diffusion passage length give the nanofiber drug system higher overall release rate than the bulk material (e.g. film).

Background; Electrospinning Technique

- Biomedical Applications; Drug Delivery
  - Many factors may influence the release performance, such as
    - Type of polymers used.
    - Hydrophilicity and hydrophobicity of drugs and polymers.
    - Solubility.
    - Drug polymer comparability.
    - Additives, and the existence of enzyme in the buffer solution.

Presentation Outlines

- Encapsulation of Cells into Electrospun Nanofibers
  - Introduction
  - Examples

Encapsulation of Cells into Electrospun Nanofibers

- **Biohybrid materials**: containing or composed of both biological and non-biological components.
- The ability to electrospin scaffolds of living organisms will be useful for the development of novel bioengineering to medical applications.
- Recently, there has been a greatly increased interest in using bacterial viruses as an alternative to bacterial antibiotics and as vectors for gene delivery (viral and non-viral vectors)

W. Salalha, J Kuhn, Y Dror and E Zussman. Nanotechnology 17 (2006) 4675–4681

Encapsulation of Cells into Electrospun Nanofibers

- Generally, biological material has been encapsulated in electrospun fibers.
- **For example;**
  - DNA has been encapsulated for potential therapeutic applications in gene therapy.
  - Some proteins, enzymes and small molecules have also been embedded in electrospun nanofibers.
  - Filamentous bacterial viruses suspended in a polymer solution were Electrospun.

Encapsulation of Cells into Electrospun Nanofibers

- The encapsulation of biological material while preserving its activity is important for many applications.
- **Challenge:**
  - The conditions of the electrospinning process that allow the encapsulation of intact bacteria and bacterial viruses while maintaining their viability.
  - However, the longevity of functional bacteria is limited once they have been isolated from their native environment.

W. Salalha, J Kuhn, Y Dror and E Zussman. Nanotechnology 17 (2006) 4675–4681

Encapsulation of Cells into Electrospun Nanofibers

- Cell encapsulation aims to entrap both viable and dead cells within the confines of semi-permeable membranes.
- Encapsulated cells are useful for a variety of biotechnological applications such as;
  - microbial fuel cell (MFC) systems,
  - bioremediation particle biofilm reactors,
  - and regenerative medicine transplant cells among others.
- The goal is to find both appropriate immobilization methods and biocompatible environments.

Review; Eyal Zussman., Polym. Adv. Technol. 2011, 22 366–371

Encapsulation of Cells into Electrospun Nanofibers

- The properties of the encapsulation materials need to be considered;
  - The surface texture,
  - The mechanical and chemical stability,
  - The permeability of the materials used to construct membranes.

    Their permeability is very important and must allow for the diffusion of nutrients required for cell growth and the by-products released.
- Moreover, the encapsulation process needs to be sufficiently gentle so as not to expose the cells to
  - extreme mechanical stress and osmotic pressure,
  - employing chemicals that do not impair cellular function.

Review; Eyal Zussman., Polym. Adv. Technol. 2011, 22 366–371

ELECTROSPINNING OF BIOHYBRID FIBERS

- The feasibility of incorporating biological materials into electrospun nanofibers and creating biohybrid fibers has been studied by several groups.
- The biological material, for example bacteria cells, is initially dispersed in a polymer solution (Blend Electrospinning).
  - Microorganisms were entrapped into polymeric materials.
  - The electrical charges are mainly distributed at the free surface of the jet, and therefore the encapsulated biological objects are not affected electrically.

Andrea Townsend-Nicholson and Suwan N. Jayasinghe. Biomacromolecules 2006, 7, 3364-3369

ELECTROSPINNING OF BIOHYBRID FIBERS

- However, due to the hydrodynamic stresses in the jet, the encapsulated bacteria may be affected.
- Fortunately, viscous stress can be easily adjusted by controlling the solution' s viscosity.

- Another type of electrospinning is co-electrospinning, in which core–shell nanofibers are fabricated.
  - Biological materials have been encapsulated into electrospun and co-electrospun fibers.

Andrea Townsend-Nicholson and Suwan N. Jayasinghe. Biomacromolecules 2006, 7, 3364-3369

ELECTROSPINNING OF BIOHYBRID FIBERS

- When using electrospinning for encapsulation, and especially when working with cells, several mechanical issues should be considered.
  1) The viscous forces acting on the cells must be controlled,
  2) The rapid dehydration that is expected to occur during spinning needs to be avoided as much as possible.
  3) Toxicity issues also need to be considered with the goal of minimizing the exposure of the bacteria to organic solvents.

Andrea Townsend-Nicholson and Suwan N. Jayasinghe. Biomacromolecules 2006, 7, 3364-3369

ELECTROSPINNING OF BIOHYBRID FIBERS

- When using electrospinning for encapsulation, and especially when working with cells, several mechanical issues should be considered.
  4) From a morphological point of view, the porosity of the fiber matrix must be controlled to facilitate the transfer of small molecules into and out of the fibers, while taking into consideration the hydrophobicity of the matrix.
  5) Geometrical confinement issues which can affect the bacteria after solidification of the fibers should be addressed.

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Andrea Townsend-Nicholson and Suwan N. Jayasinghe. Biomacromolecules 2006, 7, 3364-3369

ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

- Monolithic nanofibers are those made from a single droplet.
- In many cases a non-aqueous polymer solution is used, and thus the main question is whether the embedded agent will remain either *viable or retain its biological properties after encountering non-aqueous solvents*.
- To overcome the problem of solvent toxicity, water-soluble polymers can be used.

Andrea Townsend-Nicholson and Suwan N. Jayasinghe. Biomacromolecules 2006, 7, 3364-3369

ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

- **In 2006, W. Salalha *et al.*** demonstrated the encapsulation of whole organisms, both viruses (T7, T4, λ) and bacteria (*Escherichia coli*, *Staphylococcus albus*), into poly(vinyl alcohol) (PVA) electrospun nanofibres.
- Electrospinning was carried out by making a suspension of bacteria or viruses in the polymer solution (PVA; 14%w/w).
- The bacteria or the virus were dispersed in the dilute salt solution or Luria–Bertani (LB) media.

W Salalha, J Kuhn, Y Dror and E Zussman., Nanotechnology 17 (2006) 4675–4681

ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

- Electrospinning parameters;
  - The flow rate = 0.2–0.5 mL h^{-1}.
  - The strength of the electrostatic field was 1.1 kV cm^{-1}.
  - TCD = 12 cm.
  - The linear speed at the edge of the disc collector was V = 8.8 m s^{-1}.
  - At room temperature (~24°C), and a humidity of about 50%.

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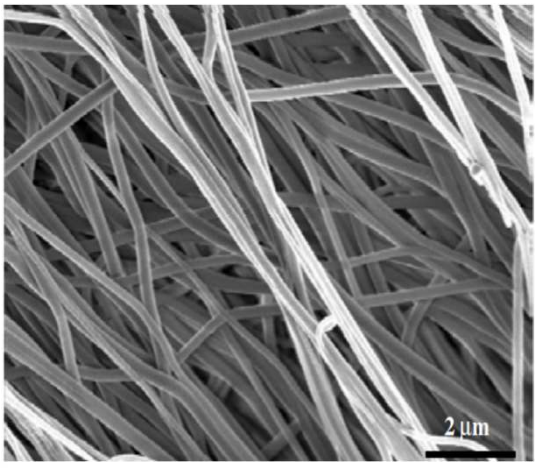
ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING



HRSEM micrograph of a mat formed by electrospun PVA nanofibers *S. albus* cells.

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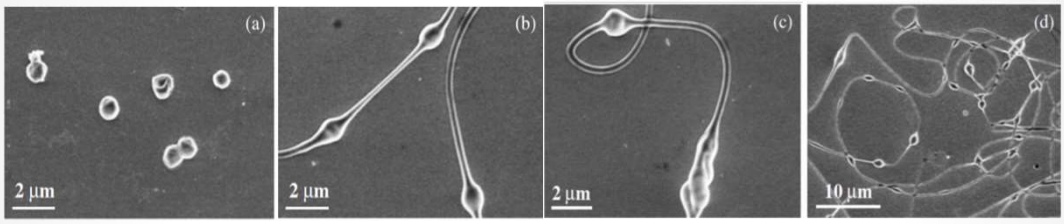
ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

(a) individual *S. albus* cells (b)–(d)) embedded *S. albus* cells in electrospun PVA nanofibres.



(c) aggregate of several bacterial cells. (d) A lower magnification of these fibers.

- The electrospun nanofibers had a
  - diameter ranging between 250-400 nm
  - uniform thickness without the formation of beads.

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BLENDING ELECTROSPINNING

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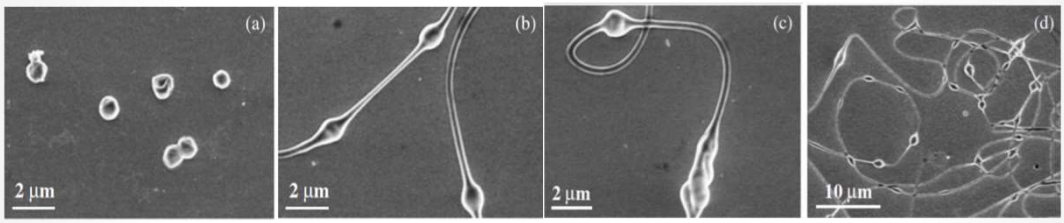

(c) aggregate of several bacterial cells. (d) A lower magnification of these fibers.

- The *S. albus* cells are distributed along the as-spun nanofibres and the average distance between them is 6±2 µm.
- In some places an aggregation of cells within the nanofibers is observed.

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BLENDING ELECTROSPINNING

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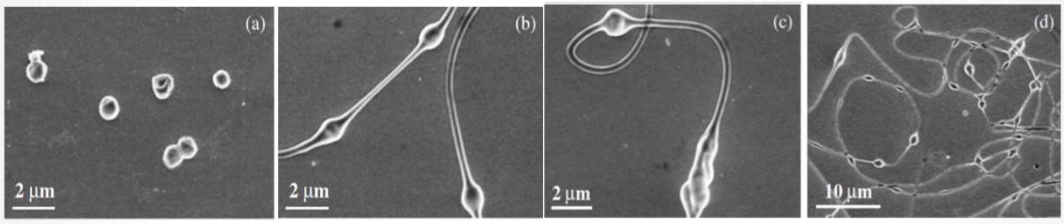

(c) aggregate of several bacterial cells. (d) A lower magnification of these fibers.

- Such aggregates were also observed before spinning and it is apparent that the electrospinning process has not disrupted these aggregates.

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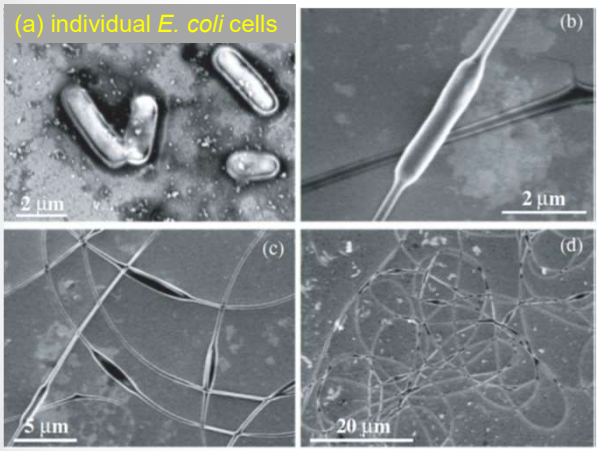
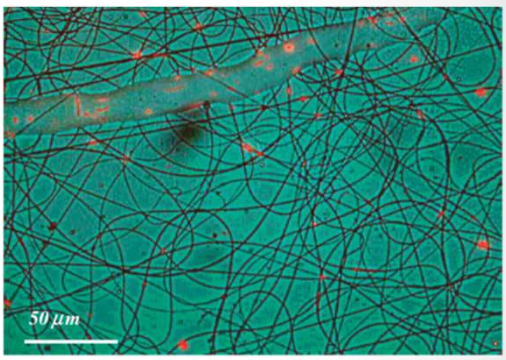
ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING



HRSEM micrographs of (a) individual *E. coli* cells, and ((b)–(d)) embedded *E. coli* cells in electrospun PVA nanofibres. (d) A lower magnification of these fibres.



An image of fluorescent *E. coli* cells (the red spots) embedded in electrospun PVA-polymer nanofibres. Both a large fiber and individual nanofibers with embedded fluorescent cells are shown.

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ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

Immediately after electrospinning, the viability was found to be:

	E. coli	*S. albus*	T4	T7	Lambda
Viability%	19	100	1	2	6

Viability of electrospun bacteria and bacteriophage. The numbers represent the relative viability (viability after spinning/viability before spinning).
All organisms were suspended in LB before spinning. The sources of error are the weighing of the spun material, the dilution steps and the plating error. The total error is estimated to be between 20 and 40%.

Both the Gram +ve *S. albus* and the Gram -ve *E. coli* have strong cell walls and can withstand at least 50,000x the force of gravity in high speed centrifuges without effect.

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ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

- Experiments were carried out with *E. coli* to determine whether survival during electrospinning could be improved.
  - Cells grown in Vogel–Bonner minimal medium were much more susceptible to death during the electrospinning process than those grown overnight in LB.
  - Cultures of *E. coli* grown in Vogel–Bonner medium and then washed with 10% glucose, sucrose or and suspended with the same sugar were also examined.

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ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

Viability of *E. coli* suspended in different solutions. The bacteria were placed in different solutions before spinning and viability was assessed directly after spinning and compared to that before electrospinning. The numbers represent of the relative viability. The sources error are the weighing of the spun material, the dilution steps and the plating error. The total estimated to be between 20 and 40%.

	5% glycerol	10% glycerol	10% sucrose	10% glucose
Viability%	48	22	0.2	0.07

- Only glycerol gave a substantial increase in viability after electrospinning.
- Glycerol enters *E. coli* by facilitated diffusion without chemical modification and may protect the cells from the rapid dehydration that is expected to occur as the nanofibres are generated which may be the reason for the relatively low viability of *E. coli*.

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ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

VIABILITY AFTER ELECTROSPINNING

- After the organisms were embedded in fibers, they were stored at room temperature, 4, −20 or −55°C and the viability of the stored material was periodically examined.
  - After one month at room temperature

    Both bacterial species showed a complete loss of viability
- Some loss at 4°C during 3 months (*S. albus*) and 4 months (*E. coli*).
- Viability were essentially completely stable at −20 and −55 °C.

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ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

- Generally,
  - It was shown that bacterial viruses and bacterial cells remain viable after spinning.
  - *S. albus* remained completely viable.
  - *E. coli* cells showed an 81% reduction in their colony-forming ability; however, this could be improved to about 50% when the cells were suspended in 5% glycerol prior to spinning, thus avoiding rapid dehydration.
  - Also, it was found that in the fibers all organisms retain their viability at -20 and -55°C for at least 3 months without further loss.

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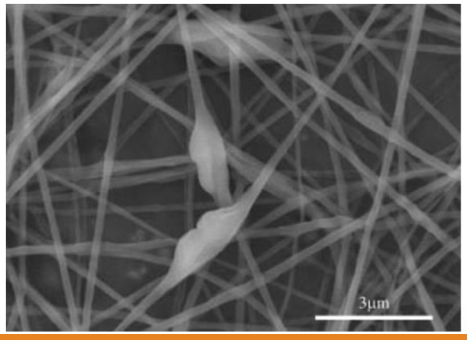
ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

- Another application of PVA dissolved in water to fabricate biohybrid fibers was demonstrated by Lopez-Rubio *et al.*
- He used it for the encapsulation of probiotic bacteria (*good for your health, especially your digestive system*).
- Their study showed that living cells of the strain *B. animalis* Bb12 can be successfully encapsulated in fibers with the cells retaining their viability for 40 days at room temperature and 130 days under refrigeration.

A. Lopez-Rubio, E. Sanchez, Y. Sanz, J. M. Lagaron, Biomacromolecules, 2009, 10, 2823.

ELECTROSPINNING OF BIOHYBRID FIBERS;
BLENDING ELECTROSPINNING

- Gensheimer *et al.* demonstrated the potential for live cell encapsulation by producing electrospun polyethylene oxide (PEO) nanofibers in water; however, they found considerable variability in bacterial viability between *M. luteus* and *E. coli*.



SEM image of composite electrospun fibers consisting of PEO and *M. luteus*.

M. Gensheimer, M. Becker, A. Brandis-Heep, J. H. Wendorff, R. K. Thauer, A. Greiner, Adv. Mater. 2007, 19, 2480.

ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- Core–shell nanofibers can be produced by the co-electrospinning of two different solutions at the same time.
- Electrospun core–shell polymeric microfibers offer a robust method for bacterial cell encapsulation.
- In this process,
  - The biological material is dispersed in the inner polymer which is immobilized within a solid outer matrix.
  - The solid matrix serves as a shell surrounding the "fluid" compartment, i.e. the core.

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ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- The shell supplies
  - The mechanical strength
  - Chemical stability (e.g. insoluble in water),
  - And supports the transport of matter into and out of the compartment.
- Well stabilized co-electrospinning process can be achieved when
  - Both solutions have the ability to be spun on their own
  - The core and shell solutions are immiscible.
- Also, core–shell nanofibers made of miscible solutions can be achieved.

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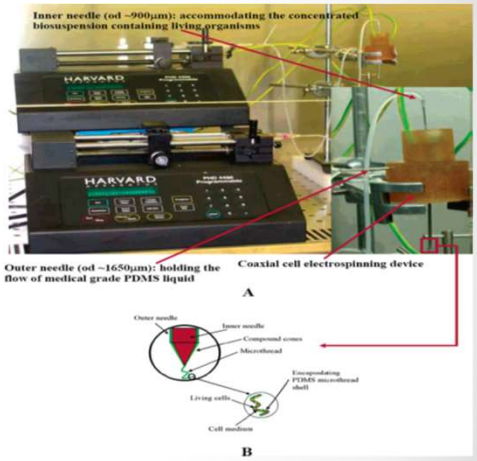
ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- In 2006, Jaysinghe and Townsend encapsulated mammalian cells (cell line 1321LN) by using a coaxial needle arrangement with the concentrated biosuspension in the inner needle and medical grade polydimethylsiloxane (PDMS) in the outer needle



(A) Coaxial cell electrospinning device setup, showing the flow inlets for the biosuspension and PDMS media.
(B) (B) A schematic representation of the generated thread.

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ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- Cell viability

Threads were collected for

(1) analysis of the electrospun cells

Threads were collected in growth medium-containing Petri dishes.

Aliquots of cells were mixed with Trypan Blue (azo dye) solution at a final concentration of 0.2%, and live (clear) and dead (blue) cells were counted within 2 min of sample preparation by use of a hemocytometer.

(2) analysis of the electrospun fibers.

% cell viability = [(unstained living organisms)/(total organisms)] X100

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ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- The viability of cells passed through the electric field (67.6% ± 1.9%) was not statistically significantly different from the viability of control cells (70.6%±5.0%) that were collected at the same flow rate but without an applied voltage.

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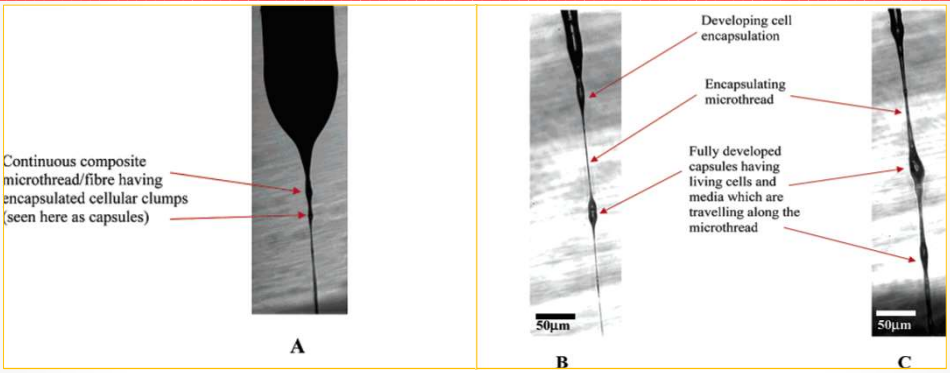
CORE–SHELL NANOFIBERS



(A) Stable cell electrospinning, showing capsules generated by cell clumping within the suspension, just below the cone apex.

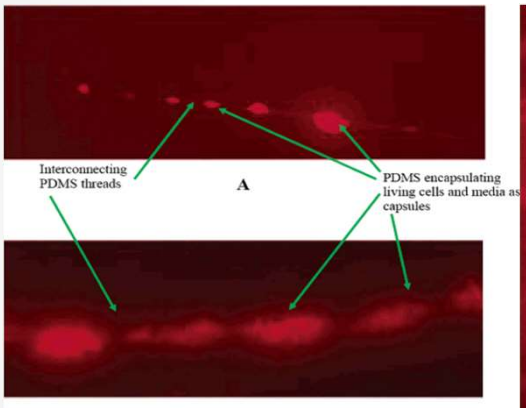
(B) Cell electrospinning of living cells at the flow rate condition "E6".

(C) Electrospinning of living organisms using the flow rate condition "A2".

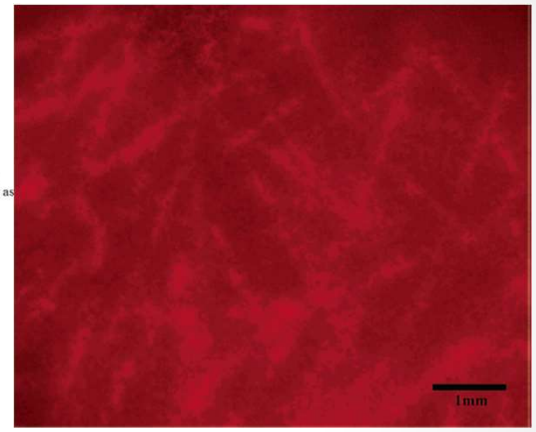
Characteristic high-speed photographs; The formed fibers near collection show a pellet-like cellular encapsulation seen as a cluster within the biological microthread. Both (B) and (C) were carried out at an applied voltage of 9.5 kV.

Andrea Townsend-Nicholson and Suwan N. Jayasinghe. Biomacromolecules 2006, 7, 3364-3369

ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS



Characteristic fluorescent micrographs showing the variation in fiber diameter that results from cell encapsulations. Experiments were conducted at an applied voltage of 9.5 kV under flow rate conditions E6 (A) and A2 (B).



Fluorescent micrograph of a nylon-membrane after collection of a fluorescently labelled electrospun biosuspension. The pattern seen is representative of that obtained in multiple experiments and shows the biologically active scaffold that is comprised of composite threads that contain the living organisms in media.

Dr. Mohamed El-Newehy
Andrea Townsend-Nicholson and Suwan N. Jayasinghe. Biomacromolecules 2006, 7, 3364-3369

ELECTROSPINNING OF BIOHYBRID FIBERS;
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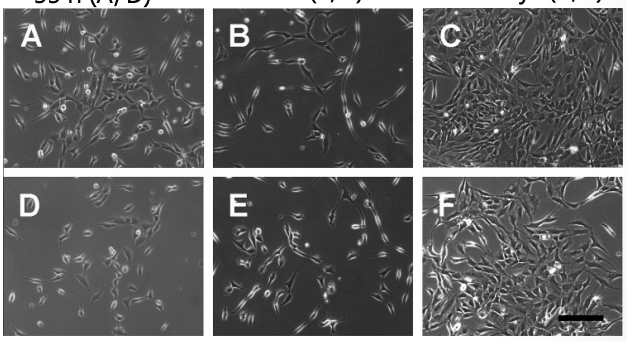
- Cells were examined for their morphology and for their rate of growth.

Photomicrographs shown here were taken at
55 h (A, D) 80 h (B, E) 6 days (C, F)

Control cell cultures (Figure 6A-C)

Electrospun cell cultures (Figure 6D-F).



- There was no observable difference in either of these attributes over the period of study

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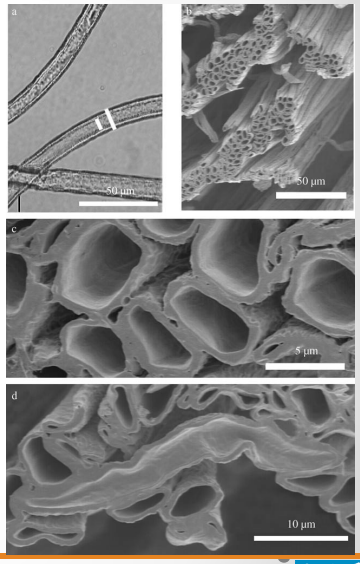
ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- The work of Dror *et al.* demonstrated that proper selection of the polymer shell and core system can result in polymeric microtubes, i.e. hollow fibers.
- In this process the shell solidifies while the solvent in the core solution evaporates, leaving a polymer residue which is adsorbed to the inner side of the shell, thus creating a microtube.
- The fabricated microtubes were found to be effective for encapsulating enzymes.



Y. Dror, W. Salalha, R. Avrahami, E. Zussman, A. L. Yarin, R. Dersch, A. Greiner, J. H. Wendorff, Small 2007, 3, 1064

ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- The induced pores in the shell had a pronounced effect on the movement of molecules into and out of the microtubes.
- An enzyme of about 80 kD, alkaline phosphates (AP), slowly diffused through the pores into the surrounding medium.
- However, a larger enzyme, β-galactosidase (520 kD), remained in the fibers without any leaching.

 Y. Dror, W. Salalha, R. Avrahami, E. Zussman, A. L. Yarin, R. Dersch, A. Greiner, J. H. Wendorff, Small 2007, 3, 1064

ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- In 2009, Klein *et al.* reported the encapsulation of bacterial cells in electrospun microtubes.

  i.e. Co-electrospinning of a core polymeric solution with bacterial cells and a shell polymer solution resulted in microtubes with porous walls.

- Preparation of Polymer Solutions:
  - The shell solution was 9wt% polycaprolactone (PCL) + 1wt % polyethylene glycol (PEG) 6 K dissolved in a mixture of chloroform and dimethylformamide (DMF), 90:10 (w/w).
  - The core solution was 5wt % polyethylene oxide (PEO) in H_2O.

 Klein, J. Kuhn, R. Avrahami, S. Tarre, M. Beliavski, M. Green, and E. Zussman. Biomacromolecules 2009, 10, 1751–1756

ELECTROSPINNING OF BIOHYBRID FIBERS;
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- Cells were suspended in the core solution and via co-electrospinning were encapsulated within the microtubes.
- The spinning parameters were as follows:
  - The electrostatic field used was approximately 0.7 kV cm^{-1}.
  - The distance between the spinneret and collector plate was 14 cm.
  - The flow rates of both the core (0.5 mL/h) and shell (3.5 mL/h) solutions were controlled by two syringe pumps.

 Klein, J. Kuhn, R. Avrahami, S. Tarre, M. Beliavski, M. Green, and E. Zussman. Biomacromolecules 2009, 10, 1751–1756

ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- Bacterial Strains and Growth Conditions.
  - *Escherichia coli;* (can grow on lactose and resistant to the antibiotic kanamycin).
  - *Pseudomonas* ADP; (grow with the herbicide atrazine as the sole source of nitrogen and synthesizes an alkaline phosphatase whose activity can be measured in whole cells).
  - *Pseudomonas putida; red fluorescent protein (DsRed)*

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ELECTROSPINNING OF BIOHYBRID FIBERS;
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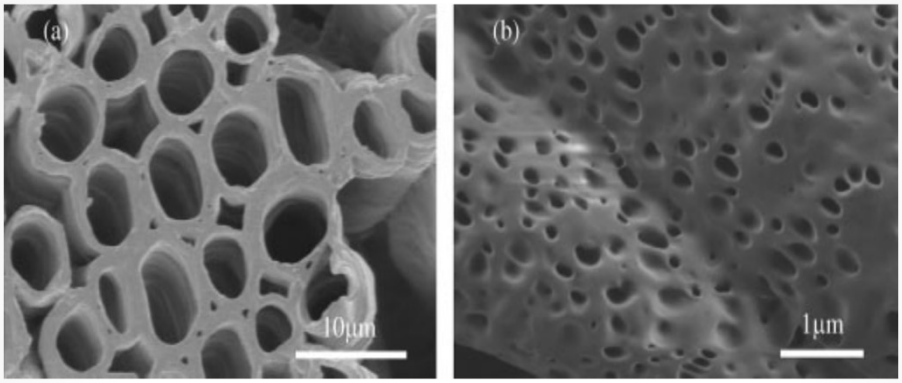

Cross-sections of the microtubes and their surface morphology; Images of hollow as-spun fibers. (a) a cross-section of several microtubes, and (b) an image of the free surface of the fibers showing the pores.

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ELECTROSPINNING OF BIOHYBRID FIBERS;
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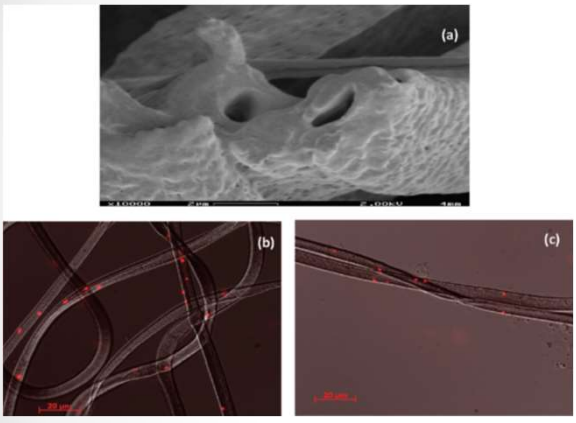

(a) HRSEM micrographs of microtubes used for encapsulating living cells; (b,c) fluorescent microscopy images of encapsulated *P. putida* S12:dsred.

- The resultant fibers are partially collapsed microtubes with porous walls which are due to PEG in the shell solution.
- The radial collapse of the microtubes is expected for this combination of core-shell solutions.
- This collapse can occur to such an extent that the microtubes are transformed into a ribbon like structures.
- The tubular space available for the encapsulated bacteria seems rather restricted, averaging a diameter of 3.5 μm.

 Klein, J. Kuhn, R. Avrahami, S. Tarre, M. Beliavski, M. Green, and E. Zussman. Biomacromolecules 2009, 10, 1751–1756

ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- Encapsulated Cell Viability.
  - These studies demonstrate the feasibility of novel Electrospun microtubes as a method to immobilize bacterial cells.
  - Determining cell viability directly is difficult for encapsulated microbial cells in Electrospun core-shell fibers.
  - Due to the insoluble character of the microtube, a direct assessment of cell viability by the usual plate counting technique is not possible.
  - The physiological properties of encapsulated cells were, therefore, evaluated by cell respiration and their ability to synthesize proteins.

 Klein, J. Kuhn, R. Avrahami, S. Tarre, M. Beliavski, M. Green, and E. Zussman. Biomacromolecules 2009, 10, 1751–1756

ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- Encapsulated Cell Viability.
  - Bacterial cell encapsulation combined with the ability to control mass transfer through the microtube shell creates a bioreactor like structure.
  - In addition, this fabrication method enables encapsulation of different kinds of bacterial cells in the same microtube.
  - This technique has the unique benefit of a large ratio of surface to volume and should find use when separation between bacterial cells and the external aqueous environment is desired (such as in water purification processes).

Dr. Mohamed El-Newehy Klein, J. Kuhn, R. Avrahami, S. Tarre, M. Beliavski, M. Green, and E. Zussman. Biomacromolecules 2009, 10, 1751–1756

ELECTROSPINNING OF BIOHYBRID FIBERS;
CORE–SHELL NANOFIBERS

- Encapsulated Cell Viability.
  - The as-spun microtubes can be easily integrated into water purification systems.
  - Microtube technology also offers significant advantages for studying the behavior of cells under confinement.
  - Cells, either bacterial or mammalian, were found to alter their behavior in a confined environment.

 Klein, J. Kuhn, R. Avrahami, S. Tarre, M. Beliavski, M. Green, and E. Zussman. Biomacromolecules 2009, 10, 1751–1756