Developing of

Nano-Micro Tubes & Fibers for Biomedical Applications

Ali Ghasemi Azar

M.Sc. Thesis

Master of Science in Physics

Supervisor:

Professor Martin Kanje

Department of Biology and Physics

Lund University

August 2013

Reflection

Professor Michio Kaku is a theoretical physicist and co-founder of string field theory. He is the author of several scholarly textbooks and more than 70 published articles covering various topics like superstring theory, supersymmetry, supergravity and hadronic physics. Michio Kaku is also a futurist. He believes in different types of civilizations. For Michio, type 1 civilization **"***is truly a planetary society, who has mastered all forms of terrestrial energy. Their energy output is much greater than ours***"**. And he continues:
"*We are Type 0**.*** *We still get our energy from dead plants. Pretty pathetic, if you ask me. I could only imagine what an advanced alien civilization thinks of us. With our racism, wars, and class struggles we will be lucky if we ever get to a Type 1. At the current rate, in my opinion the human race is headed toward extinction. The generation now alive and our grandchildren are the most important generations ever to walk the surface of the Earth. We are the generations that will determine whether we make the transition from Type 0 to Type 1 or we destroy ourselves because of our arrogance and our weapons."* **Michio Kaku**

Humanity! The unexpressed information!

What makes us human is the information stored in our DNA. All types of manmade data storage devices like CDs, DVDs, Hard drives and flash memories have a limited storage capacity and short lifetime. DNA is the most durable storage device ever known to man and can preserve stored information over cosmological time scale. Only 1 gram of DNA can store around 700 TB (Terabyte) of information. For me this is amazing! Perhaps the concept of the life coded in DNA has traveled over cosmological distances through the space before seeded on the earth.

It is known that in addition to the social and environmental effects, our behavior and personality also have some roots inside us, in our genes! Perhaps we represent only a part of the information that is stored in our blueprint! I think an important part of this information has not been fully expressed in our species yet! And perhaps that information is related to the humanity! The appearance, language, nationality**…** and even the name of the people still dominate the humanity! Technology is advancing! And we are not perfect! This imperfection led us to develop technologies that can wipe up our species and perhaps all the life on the earth forever. This is a very critical point in time for our species! Will we be able to make the transition from Type 0 to Type 1 civilization? It seems challenging! However I think there is a glimmer of hope! And this hope comes from neuroscience. Stimulation of the brain along with recording the neural activities has opened a new window in neuroscience for not only the treatment of the movement disorders, control of the prosthesis, reducing the pain and depression but also in few decades we maybe witnessing the creation of the brain networks! Mass information can be stored, shared and processed in the brain networks! This will have a great impact on the education and learning methods. Knowledge will be downloaded, uploaded or shared in these biological networks in high speeds! And once these networks are developed then transition from type 0 to type 1 civilization will be inevitable! This will be the beginning of a new era that the hidden information in our blueprint will be expressed fully. This may be accompanied with some physical and biological changes in mankind. He may start to rewrite, edit or create his own blueprint and master his destiny! He will be immortal!

Irresponsibility is growing! Modern-day slavery, Genocide, Man-made chaos, pollution, poverty and ignorance still are visible! But how much are we responsible for these dilemmas? Perhaps most of us will feel a great shame when we find the answer! And this feeling of shame is a positive sign of the awareness and responsibility. I invite all the scientists to shine a light on these dilemmas, by writing few sentences in the beginning of all their publications! It will raise the awareness and responsibility! It will be a step forward towards type 1 civilization!

Ali Ghasemi Azar

April 26, 2013 LUND University, SWEDEN

Contents:

About my Master's Thesis:

Summary and Outlines

Summary:

Polymer based nanometer to micrometer size fibers and tubes are the bases for a wide range of industrial and medical applications and various research branches. They are capable of guiding light, carrying electricity and liquid or exchanging heat. Two production systems were established and built. These systems enable us to produce a wide range of tiny tubes & fibers. Light, nano-micro tubes & fibers, beads, nanoparticles and biological entities and agents (e.g. cells, antibodies and nerve growth factor) were used in this master work. The main focus in this work is on **nerve regenerative implants** and **neural electrodes.** Nerve regenerative implants encourage nerve regeneration and repair in nerve-damaged patients. I was involved in prototyping a special **implant for restoring sexual potency* after removal of the prostate in prostate cancer patients. Neural electrodes have opened a new window in neuroscience for treatment of movement disorders like Parkinson and Paralysis. The main problem with metal micro electrodes is the glial encapsulation due to "foreign body reaction". Because of this problem an implanted electrode cannot remain functional in the brain tissue for a long period of time and simply fails. I suggested an **optoelectrode* that may solve the problem. [*Novel implants]

Designing neural interface electrodes capable of mass data transfer into or out of the brain is still a challenging field. I suggested a light based neural interface electrode that may provide the possibility of mass connections with the neurons. However these approaches need to be discussed and examined empirically. I strongly believe that light and biophotonics will revolutionize neuroscience in the future and will bring endless possibilities to the field!

Outlines:

A) Two production systems were established and built:

1) **Electrospinning System:** This system enables us to produce fibers, hollow fibers and beads with diameter around 10-20 nm up to few microns. I established the system at our Nerve laboratory (Nanoneuro Group - Department of Biology - LUND University) on July 2010. Since then I have been in charge of operating and developing the system and supervising students through their projects related to micro-nanofiber based scaffolds for "in vitro" experiments.
2) **Microtube production system:** This system enables us to produce wide range of tiny tubes & fibers. I built the system on February 2011 at Department of Biology - LUND University.
   Tubes and fibers can be customized according to different applications.

B) Activities and achieved products:

- ***Nano-Micron size Fibers, hollow fibers and Beads*:** A variety of polymers were spun and different types of fibers, hollow fibers and beads with diverse morphology, diameters and combinations were produced. In case of some modifications to the mentioned systems, it is also possible to produce metallic, ceramic and glass Nano-micro fibers-tubes.
- ***Aligned fibers:*** Aligned fibers in different alignment patterns (e.g. parallel, diverged, converged and concentric) were produced.
- ***Metallic long nanotubes:*** Have already been produced during this master work. They may be used as neural electrodes.
- ***Scaffolds:*** Sample scaffolds for cell differentiation, skin and bone regeneration, hollow fiber cell culturing (bio reactor), blood vessels and axonal guidance experiments were produced and demonstrated that Nano-micro Tubes & Fibers guide the Axons nicely.
- ***Nerve regenerative implants:*** I was involved in prototyping a special implant for restoring sexual potency after prostatectomy. Several sample implants have been produced. The project may be commercialized. Due to its novelty, in this report I did not put any further information regarding this implant.
- ***Vibrating nerve regenerative implant:*** This implant is based on electromagnetic manipulation. A prototype implant has been produced and I think the idea is new.
- ***Neural electrodes:*** **a)** I worked on prototyping a special neural optoelectrode. Some pilot experiments have already been done and its novelty has been confirmed by Lund University Innovation System (LUIS). Due to its novelty, I did not put any further information regarding this electrode in this report. **b)** Coating neural electrodes with polymer based nanofibers may mimic the extra cellular matrix and provide better and flexible anchoring of the electrode in the brain tissue and may result in less tissue damage and a reduction in "foreign body reaction". Such coated electrodes were implanted in rat brain at Neuro-nano Research Center (NRC)- Lund University and is still an ongoing project.
- ***Retinal repair*:** I'm involved in a joint research project between our laboratory and Ophthalmology group at Biomedical Center (BMC)-Lund University. An assembly of special scaffolds for retinal repair was produced during this master work. However since this is still an ongoing joint project, I did not put any further information regarding these scaffolds in this report.
- ***Human embryonic forebrain stem cells:*** I was involved in this joint research project between our laboratory and Ophthalmology group at Biomedical Center (BMC) - Lund University. (No further information regarding this project is given in this report)

About this report:

The ultimate aim of this report is to demonstrate the ***potential use*** of Nano-micro tubes & fibers as various scaffolds for bio applications. This report is not a quantitative report but rather an image based report. All the images and diagrams, in this report have been produced by the author. The results are presented only in the form of short texts or images. Some of the ideas, techniques, scaffolds, products and applications on nerve regeneration, neural electrodes and vision research that I worked on during this master work, are unique and the novelty of some of them has been confirmed by the Lund University Innovation System (LUIS). I will not put them in this report. They may be published in future publications. However a full list of possibilities, techniques, products and applications with a potential to be commercialized is provided in this report (Chapter 8).

ACKNOWLEDGMENTS:

Firstly I would like to express my respect and sincere thanks to my late supervisor, Prof. Martin Kanje. He introduced me to the world of Neuroscience. His directions and encouragements helped me to develop and shape my ideas. Without Martin, this work would not be possible. May he rest in peace.

Special thanks to Dr. Fredrik- Johansson for providing a working environment, support and effective discussions and his valuable feedback on the manuscript.

Special thanks to Prof. Stefan Andersson-Engels for providing Laser laboratory facilities and effective discussions on optoelectrodes and light based neural electrodes and his valuable feedback on the report and moral support.

Special thanks to Inger Antonsson for her assistance in DRG culturing and cryostat sectioning and providing biological entities, agents and chemicals.

Special thanks to Dr. Jason Beech for his valuable feedback on the manuscript. Jason has a big mind!

My thanks go to Dr. Annika Friberg and Dr. Lina Pettersson for preparing fiber coated electrodes for implantation and implanting the coated electrodes in rats respectively at Neuronano Research Center (NRC) and effective discussions.

Thanks to Stefan Sydoff for his unlimited workshop support.

My thanks go to Rita Wallen for her technical support regarding electron microscopy.

I also wish to thank Haichun Liu PHD student and Björn Thomasson M.Sc. student for their assistance at laser laboratory of the physics department.

Thanks to John Stegmayr and Patrik Bauer PHD students for cell culturing demonstration.

My thanks go to my international coordinator Prof. Tomas Brage. Thank you for all your guidance and moral support.

I also wish to thank: Dr.Ulrica Englund Johansson, Prof.Jens Schouenborg and Dr.Cecilia Eriksson Linsmeier for facilitating joint projects between our laboratory, Ophthalmology group at Biomedical Center (BMC) and Neuro-nano Research Center (NRC).

I also wish to give my thanks to Dr. Jonas Tegenfeldt who accepted my request for doing my “experimental biophysics course project” on fabricating nanofibers on May 2010. I changed an old analog monitor to a high voltage system and spun nanofibers!

My very special thanks go to my examiner Carl Erik Magnusson for his valuable feedback on the report and all his support during my study. Carl has a very big mind! He is a source of hope and moral support.

Finally, I remember my family, despite all difficulties, their love, care and support made me strong and determined to keep going. I could not make all these work without my family support.

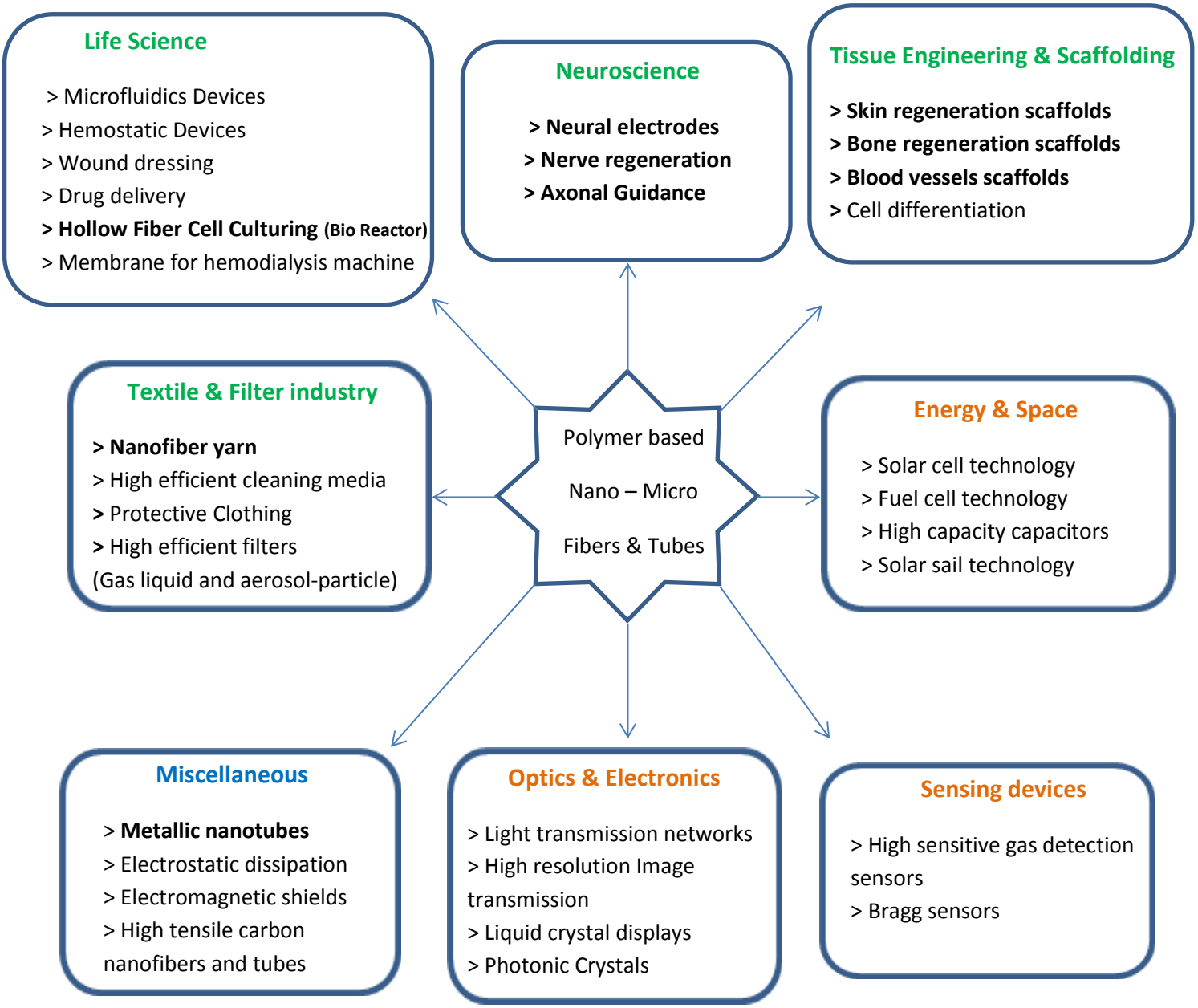
Introduction

For a long time it was believed that the properties of materials only depends on their atoms and molecules and the interconnections between them. However today we know that these properties can change remarkably when the size of a material shrinks to nanoscale dimension. This size dependency in nano dimension is due to changes that happen to the electronic properties (like magnetic properties and electrical conductivity) of the material when the size of the material is on the nanoscale. This phenomenon is known as quantum size effect that can be described by quantum mechanics. As a consequence of this effect physical properties of the materials in nano dimension will be different from what is measured in bulk state. Weak interactions like Wan der Waals or hydrogen bonds can have a quite large effect in quantum realm (where at least one dimensional size is less than 100 nanometers and quantum size effects become important). As an example, DNA with a cross section of 2nm is consisting of two helixes, and a large number of Hydrogen bonds keep them together **Ref. [1, 2].**
Quantum effect may affect the interaction between the nanofibers (diameter <100 nm) and the biological entities like axons (nerve fibers) or cells. The effect can also appear in metallic nanostructures where the surface plasmon effect becomes important. Surface Plasmon effect occurs when light hits metal nanostructures and causes electron density waves (Plasmons). These effects can appear in nanofibers and in metallic thin shell of metal nanotubes that were produced during this project. These effects are important in some special applications. However they are not discussed in this report.

By advancing the technology man is now capable of producing ultra-fine fibers & tubes from different materials like Carbon, ceramic, plastic, metal and semiconductors. This advancement has revolutionized human life and moved the technology to a new dimension. A wide range of industrial and biomedical applications are based on these tiny tubes and fibers. The key secret that makes these fibers and tubes suitable candidates for a broad range of applications lies on their high aspect ratios (surface/volume) and the possibility to make large networks. These networks are capable of carrying electricity and liquid, exchanging heat, guiding the light or releasing drugs. A large surface- to- volume ratio makes them suitable for a variety of Hi-Tech applications. Applying these networks in some potential biomedical applications is briefly discussed in this report. Enjoy!

Potential Applications

Some of the potential applications of polymer based Nano-micro tubes & fibers are summarized in the following diagram. Highlighted applications will be discussed briefly in this report.



In respect to their thickness, fibers are categorized in two groups:
Conventional fibers with typical average diameters of around 10 μm or larger
Ultra-fine fibers with a diameter from a few microns down to the nanometer scale.

Chapter 1 (*Fiber spinning*)

The aim in this chapter is to describe briefly some of the methods that are used for conventional and Ultra-fine fiber spinning.

Fiber spinning methods:

Conventional Fiber spinning

Solution spinning ***(wet, dry)*** *and* **Melt spinning** are very common methods that are being used in the textile industry. **(Fig. 1)**

In wet spinning (**Fig. 1A**) a solution of polymer is prepared and pumped through the tip of a nozzle (spinneret) with thousands of small holes. The polymer passes through those small holes, and the outcome is thin fibers. The liquid that the nozzle is placed in, will extract solvent from the polymer fibers when entering into the coagulating bath, so they start to become solid. The fibers are being pulled using some rollers (wheels) in several processes. In every stage they become thinner and thinner while losing their solvent and finally become completely solid at the end of the process. ***In dry spinning*** (**Fig. 1B**) instead of liquid, hot air is being used to evaporate solvent from the fibers.

In melt spinning (**Fig. 1C**) polymer is melted before being pumped through the tip of a spinneret. The extruded fibers are being spun by pulling them using some rollers (wheels). By changing the temperature and spinning speed, the fiber diameters can be controlled. For further information, the interested reader is referred to Ref. [31, 32]

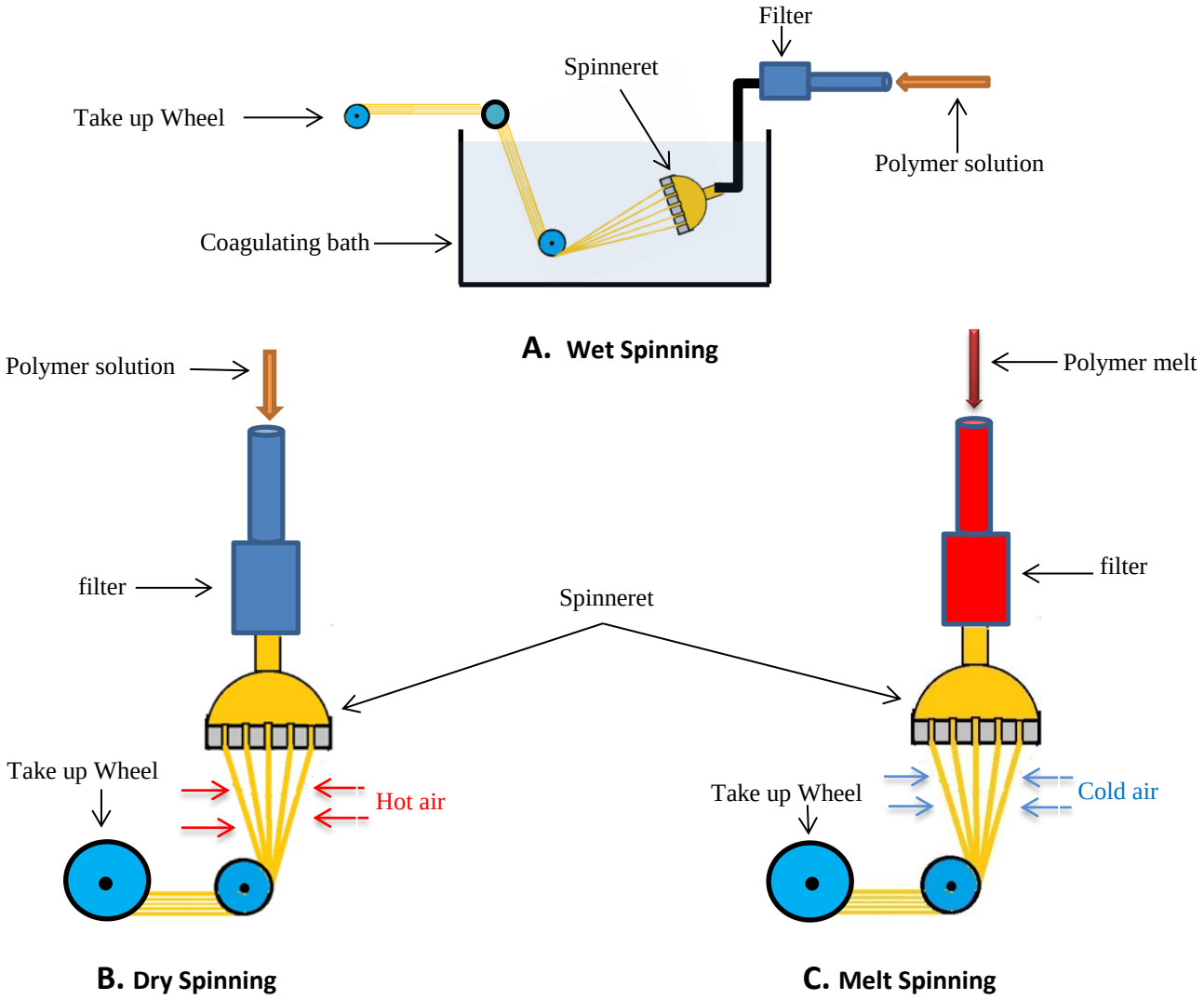


Fig.1 Conventional Fiber spinning methods

Ultra-fine fiber spinning

A large surface- to- volume ratio and the possibility to make large networks, make Ultra-fine fibers suitable for a variety of Hi-Tech applications. These fibers are produced in several different ways. *Meltblowing, Template Melt Extrusion, Electrospinning, Flash-spinning and Bicomponent spinning* are some of these methods. The first two mentioned methods will be explained briefly. However, in this report the emphasis is more on Electrospinning.

Meltblowing : The development of this technique goes back to 1950s (Naval Research Laboratory, U.S). In this processes the melted polymer exiting from an orifice of a spinneret, is elongated by injecting hot air that acts like a shield and pulls and elongates the melted polymer from tip of the orifice towards the collector and make it thinner and thinner. The resultant fibers accumulate on the surface of the collector randomly. (**Fig. 2**)
Nanofibers with diameter around 50-2000 nm have been reported **Ref. [3].**

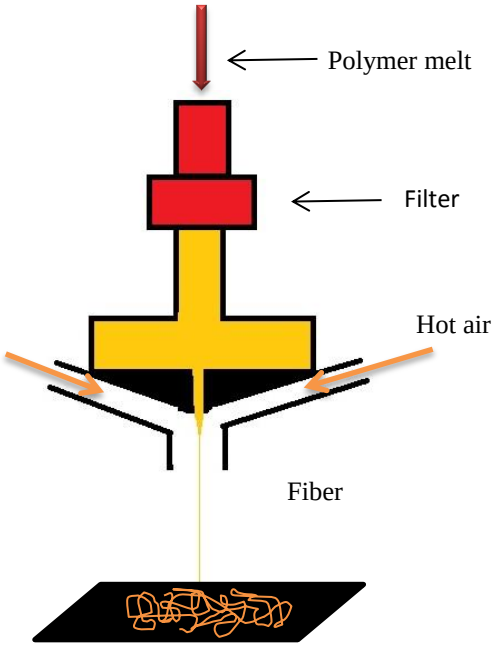


Fig. 2 Meltblowing

Template Melt Extrusion:
Anodic aluminum oxide membranes (AAOM) are used as templates. Polymer melt is pushed into template pores and then cooled to room temperature. After solvent treatment (NaOH-Ethanol) and sonication, polymer nanofibers are obtained (**Fig.3).** While the typical pore diameters of AAOM are around 200 nm; fibers with diameter from 150-400 nm have been reported. **Ref. [4, 29]**

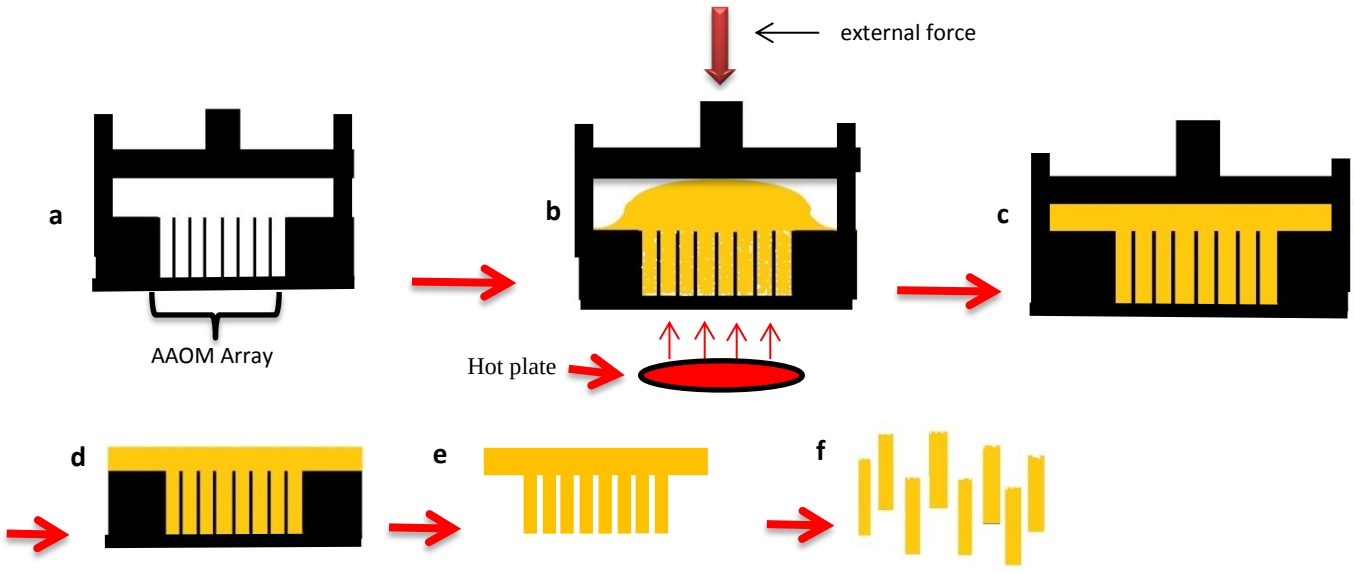


Fig. 3 Template Melt Extrusion a: AAOM is placed in sample chamber. **b:** The chamber is filled with the polymer and is placed on a hot plate. Polymer starts to melt and with the help of an external force the polymer starts to fill the pores. **c:** Pressurizing is completed and polymer fills the template and cools to room temperature. **d:** Polymer has been solidified in the chamber. However the AAOM and the polymer film that has covered the surface of the template should be removed. **e:AAOM** is removed by applying solvent (NaOH-Ethanol). **f:** Sonication is applied and Nanofibers were detached from the polymer film.

Electrospinnig:

Electrospinning is an Art! It is a very simple method for producing ultra-fine fibers. It is a unique process on nano/micron size fiber production from a polymer solution or melt. The method engages a high electric field to create nanofibers. This technique was first initiated and patented by Anton Formhals in 1934 **Ref [5].**
The ability of producing continuous submicron diameter fibers, from wide variety of materials makes electrospinning highly interesting for many research areas. Metallic, ceramic and polymeric nano-micron size fibers & tubes with diverse surface morphology and fiber orientation can be achieved through the Electrospinning technique.

In general two kinds of electrospinning systems are used; needle and needle free systems. In needle system, the flow of the polymer solution is through a needle while in needle-free system it flows from a thin layer of polymer solution. In another word, in needle electrospinning, the spinning process starts from the tip of a needle (that is a point) while in needle-free system, spinning process starts from the surface of a thin layer of polymer solution (that is an area). Although the needle-free systems are more productive, they suffer from poor control over fiber morphology and diameter. Having more control over morphology and fiber diameter in Needle electrospinning, makes it very interesting for a wide range of industrial and medical applications and various research areas and this approach is discussed further in this report **Ref [6].**

A simple theory!

In a high DC Electric field a droplet of a polymer solution or melt on the tip of a syringe needle becomes charged and then feels an electric force that changes the shape of the droplet to a cone like shape (**Taylor cone**). When the electric force exceeds the surface tension of the droplet, the droplet pulls through the electric field. In the beginning a thick stream of a solution is formed (multiple thick streams are also possible). The length of this stream can vary from a few millimeters up to a few centimeters. At some point the stream becomes unstable and charge repulsion causes rapidly whipping that leads the jet to bend and stretch. The stream becomes thin enough so that its surface tension cannot keep the whole stream intact anymore and as a result the main stream splits into thousands of narrower streams. The main stream consists of thousands of narrower streams and all of these streams have the same charge. So, as a result, the separation to the streams will take place and a jet of narrow streams will be formed. The jet starts to spin while pulling towards the cathode. Thin fibers are being formed. They get thinner and by the time they reach the cathode they are extremely thin. Resulting ultra-fine fibers are accumulated in a random way on the surface of the cathode and create a mat of random fibers. Solvent evaporates during the fiber's helical flight, in the distance between the needle and the collector **(Fig. 4 a, b & c).** For further information, the interested reader is referred to Ref. [18, 30, 33 and 40]

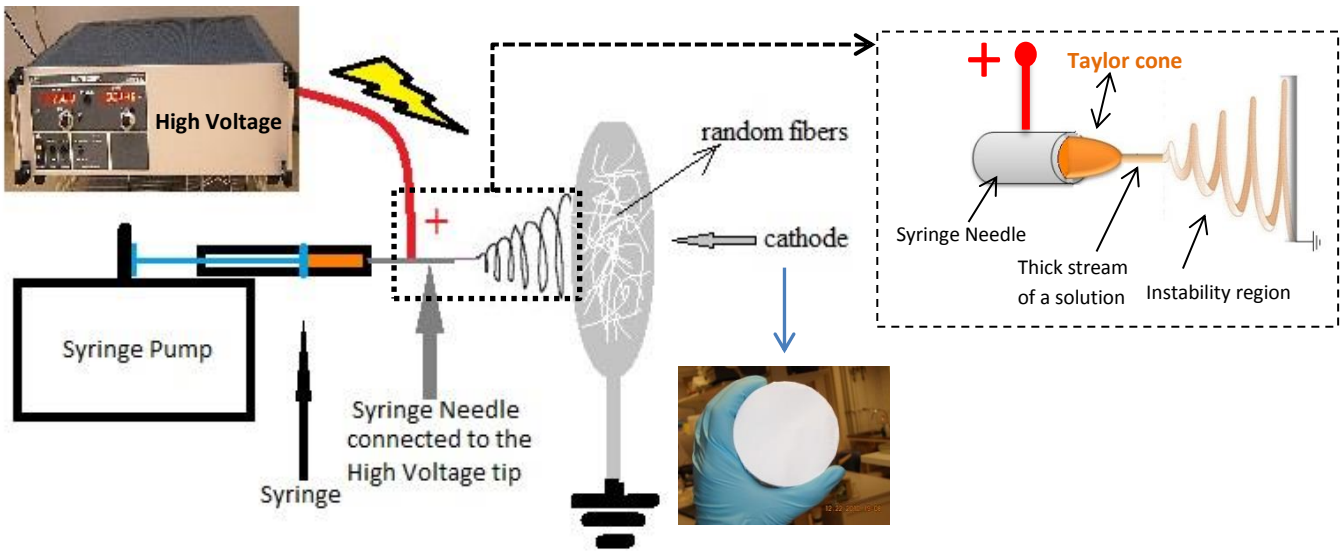


Fig. 4a

- A simple electrospinning setup!
- The white material on the cathode is a mat of Polystyrene random fibers accumulated randomly on an aluminum foil.

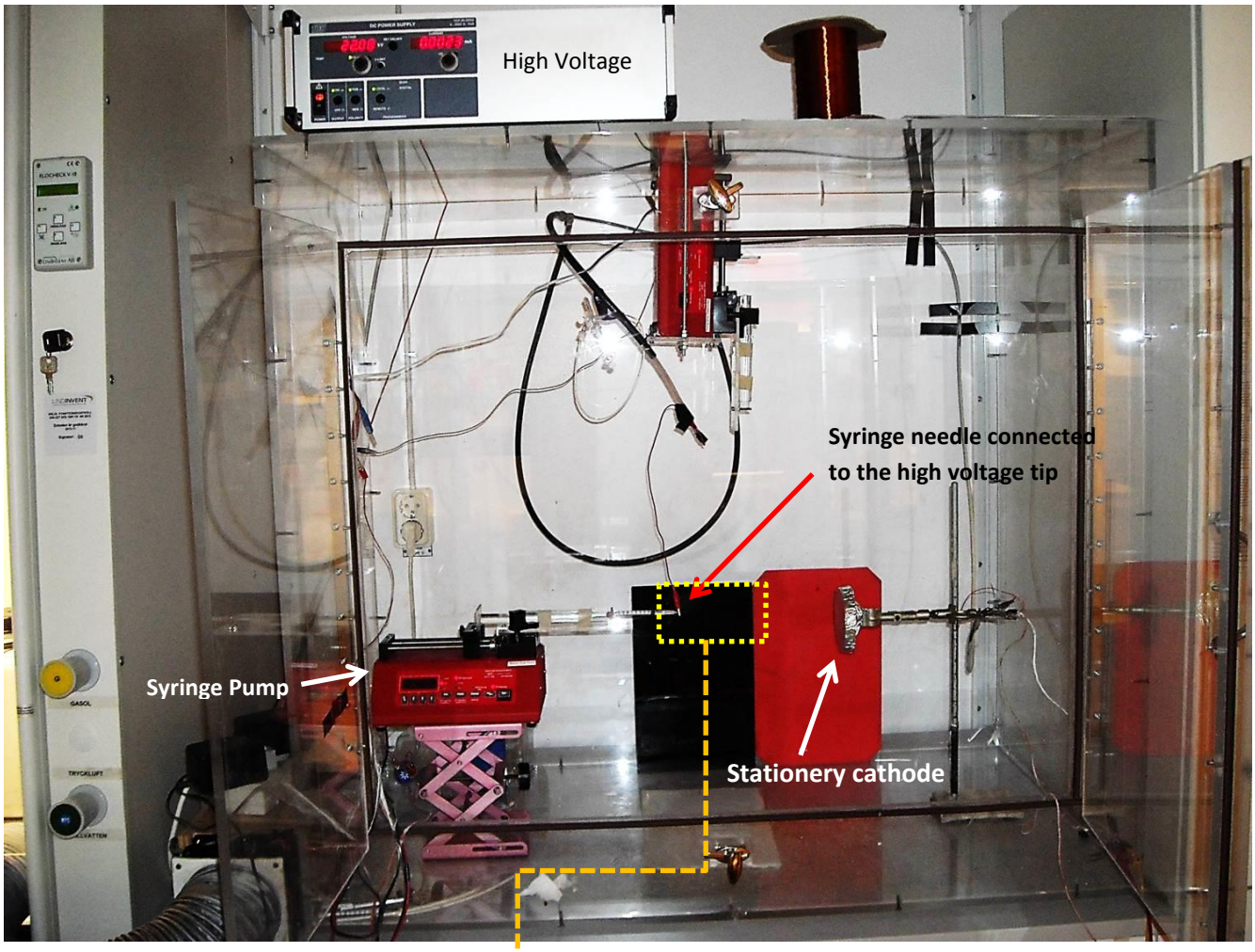
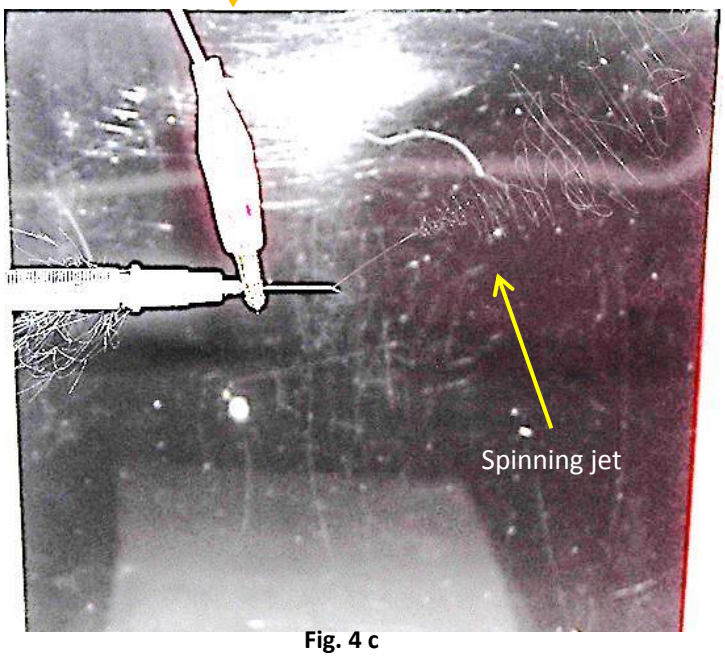
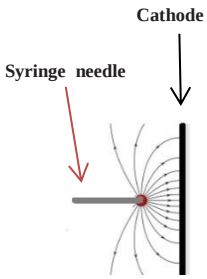


Fig. 4 b

Electrospinning system





Corresponding electric field lines

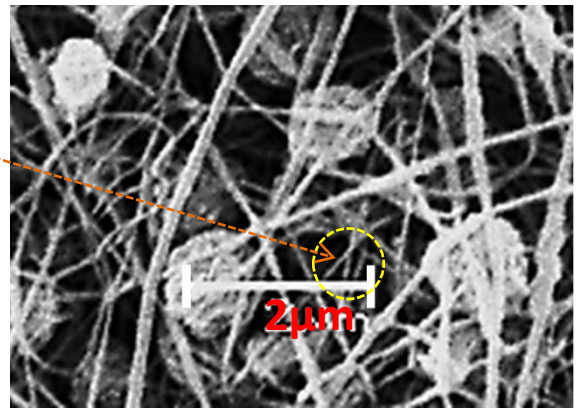
Fig. 4 c

Formation of the polymer jet

Due to charge repulsion the main stream splits into thousands of narrower streams and the jet starts to spin while pulling towards the cathode. Since the spinning jet is so sensitive to small changes in various parameters in spinning process (e.g. solvent evaporation or small voltage changes), it cannot always remain in line with the cathode and its direction changes slightly in every moment.

In respect to the polymer preparation methods, electrospinning can be done in two different processes:
Melt electrospinning and **Solution electrospinning.**
Since no solvent is used in Melt electrospinning, the process is considered clean. However due to high viscosity of molten polymer, achieving finer fibers through this process is still challenging. In solution electrospinning that was used during this project many of the solvents are toxic and this raises health and environmental concerns. However this process can lead to a diverse fiber surface morphology and sub-micron size fiber diameter. The finest fiber achieved during this project is around 10-20 nm and made of Poly-L-Lactide Acid (PLLA) fibers. (**Fig. 5)**

Fig. 5 Scanning Electron Microscopy (SEM) image of Poly-L-Lactide Acid beaded random fibers- The finest fiber diameter is around 10-20 nm.



Fiber morphology and diameter

There are nine main parameters that can affect the fiber morphology and diameter.
These parameters are categorized in three main categories:

- Solution or melt parameters:

***Concentration - Viscosity*:** An increase in concentration of the polymer solution or viscosity of the melt will lead to an increase in fiber diameter and any decrease in concentration can be resulted in finer fibers and beads **Ref [7].**
***Surface tension*:** In high surface tension, the charged droplet on the tip of the syringe needle splits into smaller droplets that can lead to formation of the beads. Reducing the surface tension will result in bead-free fibers **Ref [7].**
Conductivity: More conductive polymer solution can gain more electric charges and this will lead to a smooth spinning process and finer fibers. Conductivity of a polymer solution is increased by adding salt **Ref [8].**

- Process parameters:

Needle Diameter: increasing needle diameter will increase the solvent evaporation rate. This may lead to polymer coagulation and blockage of the needle tip. Using small needle diameter for fast-evaporating solvents is therefore recommended.
***Flow rate*:** increasing the flow rate will cause presence of more solution on the tip of the needle. This will increase the solvent evaporation rate and will lead to thicker fibers with beads. Hence at low flow rate the solvent evaporation is slow and will cause the formation of thinner fibers **Ref [8].**
***Distance*:** any changes in the distance between the needle and the cathode will alter the electric field and the jets flight time. Lowering the distance will increase the electric field and make the jet more unstable. It also will decrease the time of flight and the solvent evaporation that will lead to wet, thick and rather flat fibers with more beads **Ref [9].** Increasing the distance however, will increase the jets time of flight and hence solvent evaporation time, that will lead to finer fibers.
***Voltage*:** Increasing voltage will increase electrostatic force and will have more stretching effects on the jet. This will cause a reduction in solvent evaporation time and lead to thinner fibers **Ref [9].**

- Environmental parameters:

Temperature: Increasing the temperature, will increase the polymer solubility in solvent and will cause a reduction in viscosity. This will lead to a smooth spinning and uniform distribution of fiber diameter and will increase the solvent evaporation rate **Ref [10].**

***Humidity*:**

Humidity and solvent volatility during the spinning process are two main factors on ***fiber surface porosity***. When the solvent evaporates, solid fibers are formed. Solvent evaporation will have some cooling effect on the surface of the fibers and if the humidity of the environment is high enough, then nano-size water droplets are formed on the surface of the fibers. Droplets trace after solvent evaporation will form porosity on the surface of the fibers. This is also known as breath figures **Ref [11].**

See **Fig. 6, 7 & 8**

Fig.6. water droplets are formed on the surface of the fibers

Fig.7. Droplets trace after solvent evaporation

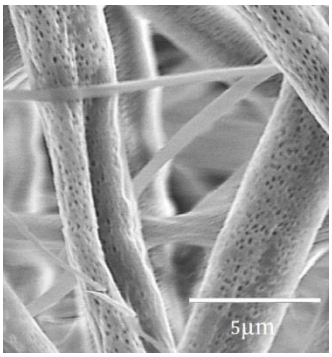


Fig.8. Poly-L-Lactide Acid porous fibers

Porosity affects fiber surface area and can be used as a parameter for controlling drug release rate from the fibers. It may also enhance the attachment of the biological entities to the fibers (*e.g.* cell-fiber or Axon-fiber adhesion).

Jet, Fiber diameter

Although electrospinning process is affected by many factors, however fiber diameter can be assumed as a function of some of the independent parameters. In a simple model presented by Fridrikh et al, jet diameter can be consider as a function of surface tension, electric current and flow rate.

In this model in an electric field a charged and viscous fluid stretches. (Fig. 8b & c)

The jet stability with respect to jet bending depends on surface charge repulsion and surface tension effects.

While surface charge repulsion causes the jet bending, surface tension tends to reduce the jet curvature and bending.

When a balance between charge repulsion and surface tension occurs, then the model suggests a limiting jet diameter:

$$D = \left(\frac{2\gamma\varepsilon F^2}{\pi I^2(2Ln\lambda - 3)}\right)^{1/3}$$

D: limiting jet diameter, γ: surface tension, ε: dielectric constant, F: flow rate
I: electric current, λ: ratio of jet length over nozzle diameter

By considering solvent evaporation during fiber formation the model relates the limiting jet diameter to the fiber diameter:

$$d = C^{1/2}D$$

d: fiber diameter, C: concentration, D: limiting jet diameter

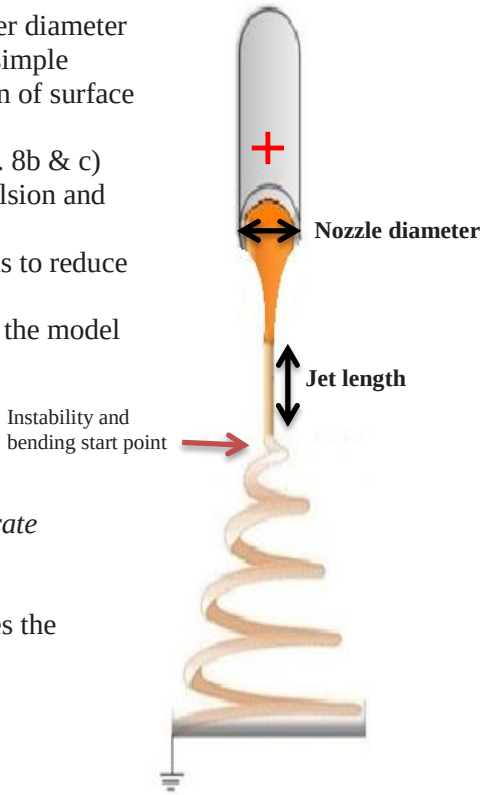


Fig. 8b. Viscous fluid stretches in an electric field

The model seems to be consistent with the experimental results. For example the model predicts an increase in fiber diameter when the flow rate increases and this is in harmony with the experimental data **Ref [38, 39].**

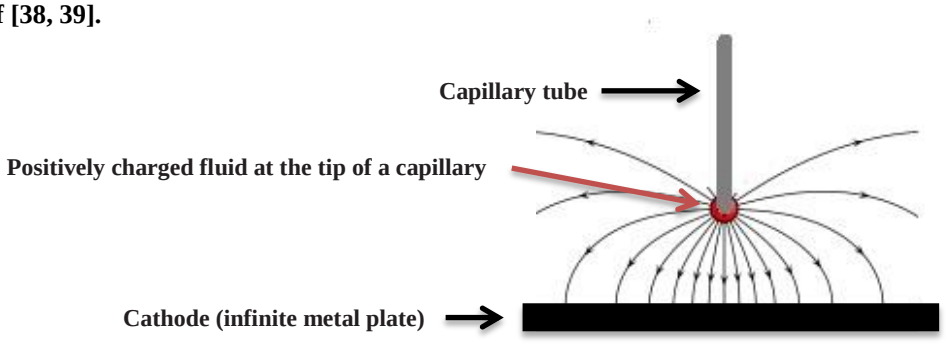


Fig. 8c. Corresponding electric field lines

From fibers to beads!

Depending on the spinning parameters, fibers, beaded fibers or just beads can be produced through the spinning process. During this project a variety of polymers were spun and different types of fibers and beads with diverse morphology and diameter were produced **(e.g. Fig. 9).**

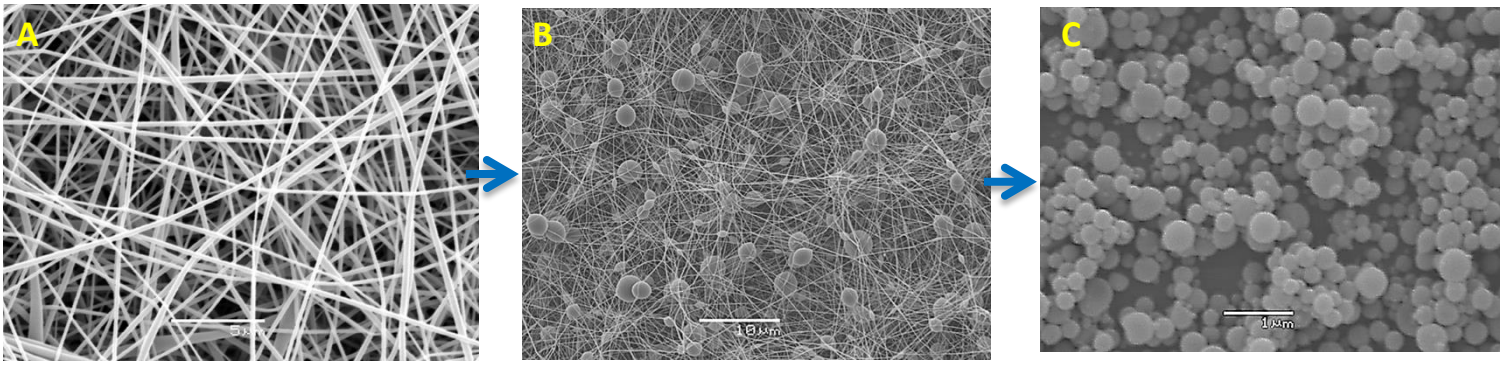


Fig.9. Polyimide fibers & beads

A. Polyimide Random fibers, B. Polyimide Beaded Random fibers, C. Polyimide beads

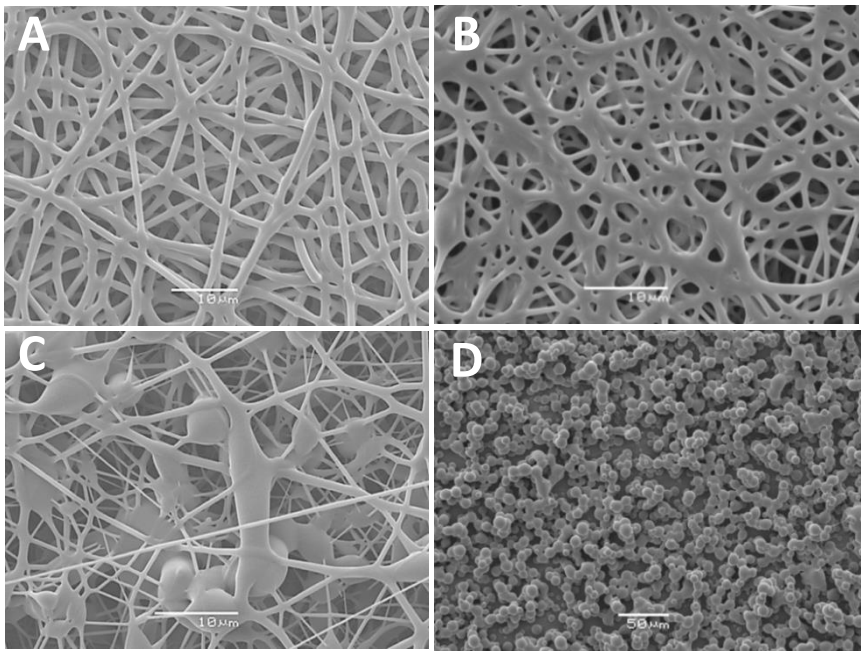


Fig.10. Poly ethylene-co-vinyl acetate (EVA) fibers & beads

A, B → Random fibers
C → Beaded Random fibers
D → Non spherical beads

Chapter 2 (*Fiber orientation*)

Along with fiber morphology and diameter, orientation of fibers is a key parameter in some specific applications. Fibers can be oriented in different ways:

- **Random fibers**
- **Parallel and concentric Fibers**
- **Converged & Diverged Fibers**
- **Custom and complex alignment**

Random fibers: Randomly oriented fiber mat is the most common case that is produced using a simple electrospinning setup. Fibers with various diameters from different polymers were produced successfully in our laboratory **(Fig.11-14).** Ultra-fine random fibers are being used in various applications including filter industry, drug delivery systems, wound dressing and in tissue engineering scaffolds. In this project they were used in a sample blood vessel scaffold and also coated on the surface of neural electrodes.

Fig.11. Randomly oriented Poly-L-Lactide Acid fibers accumulated on conductive coverslips

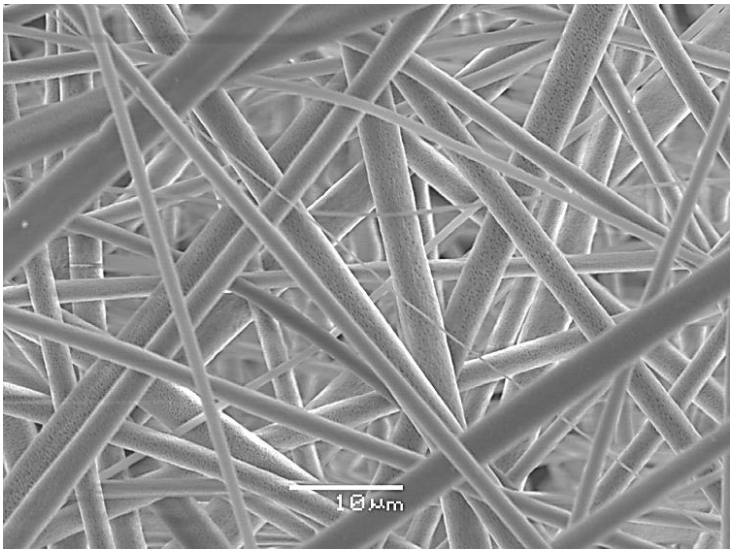


Fig.12. Randomly oriented Poly-L-Lactide Acid fibers

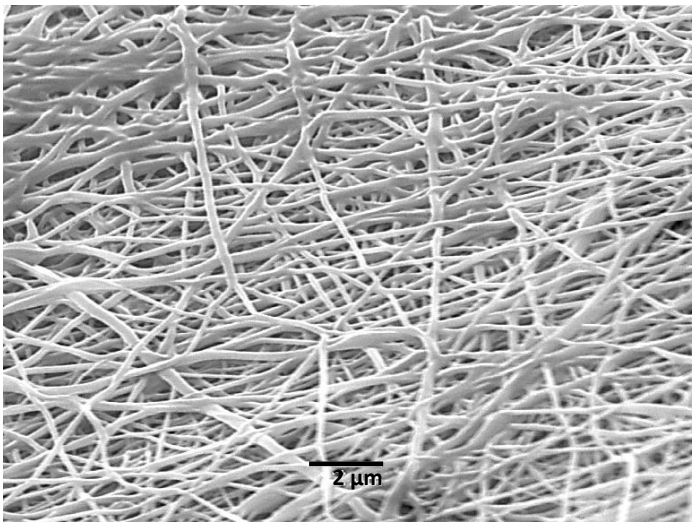


Fig.13. Randomly oriented Polyvinyl alcohol (PVA) fibers

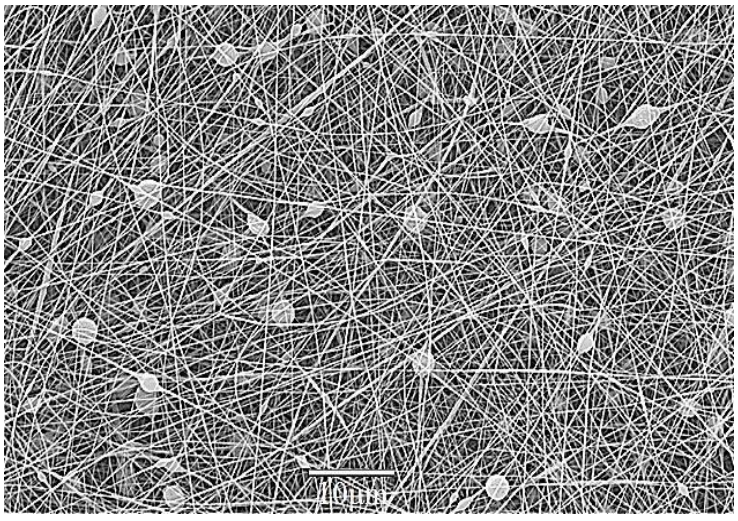


Fig.14. Randomly oriented Polystyrene (PS) beaded fibers

Parallel and concentric Fibers:

Production of parallel fibers is done using ***stationary*** **and** ***dynamic*** cathodes**:**

1. Stationary cathode,

Fibers align themselves along the gap between the two sharp edges of a U shaped cathode (**Gap method**). One end of a charged fiber attaches to one edge of the U shaped cathode while the rest of the fiber flies towards the other edge of the cathode. This causes formation of a stretched fiber across the gap. This process is repeated and creates aligned fibers. Since the rest of the fiber in every cycle feels repulsive forces from the neighboring fiber, the parallel alignment will not be perfect and every fiber will have a small angle with the neighboring fiber resulting in a zigzag alignment. **(See fig. 16)**

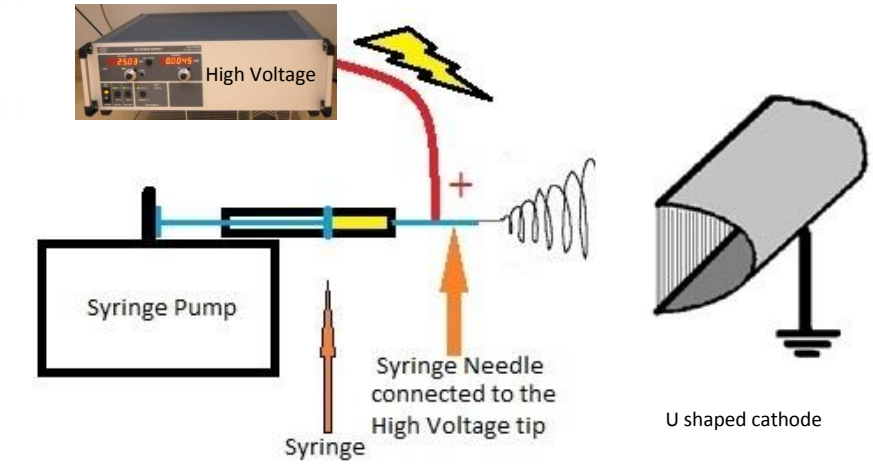
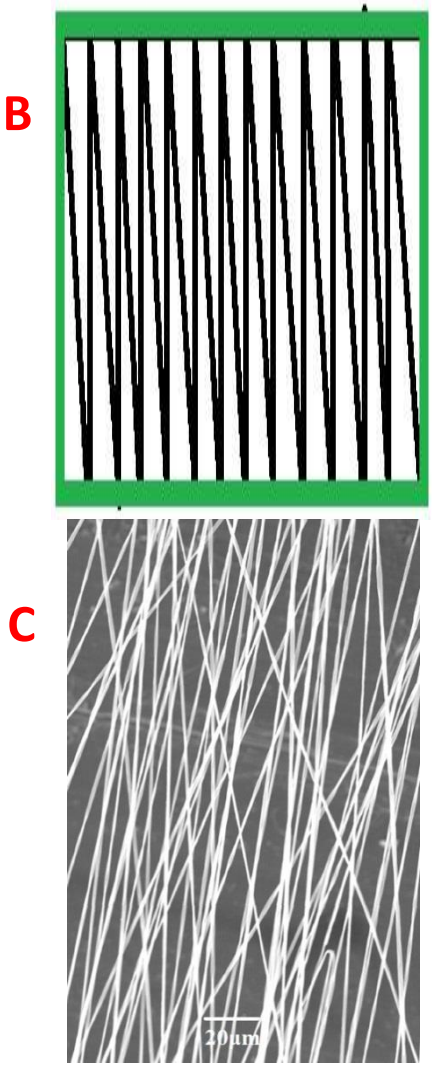


Fig15. Electerospinning with Stationary cathode



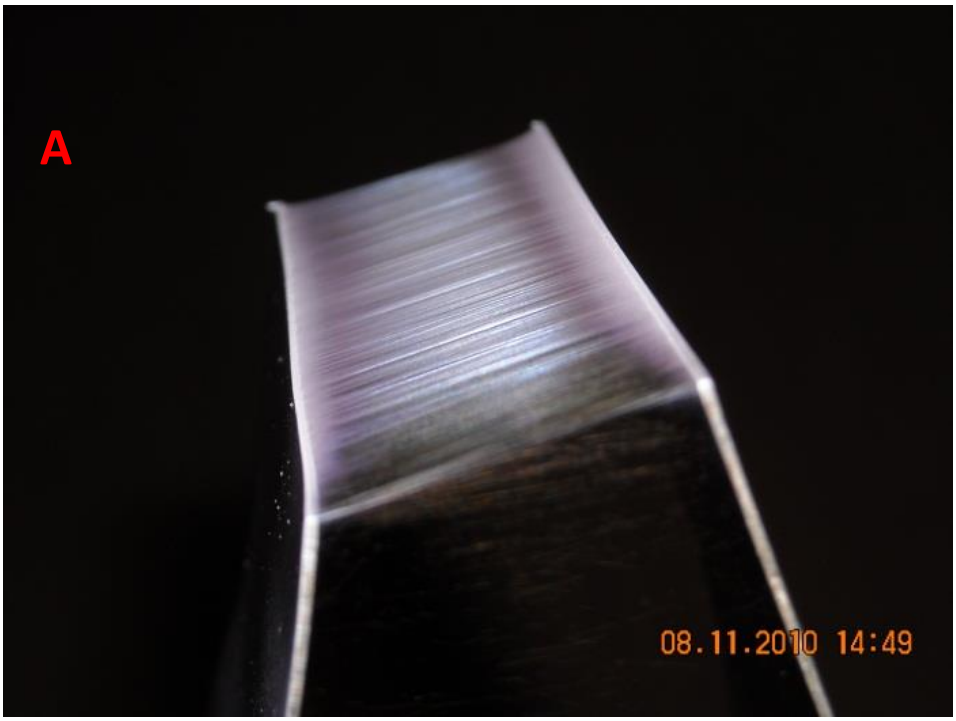


Fig.16. Aligned fibers using gap method

A) U shaped cathode with Poly-L-Lactide Acid aligned fibers between the gap

B) Zigzag pattern of fibers

C) SEM image of aligned Poly-L-Lactide Acid fibers

2. Dynamic cathode

Cathode is rotating and the fiber alignment is achieved due to high speed rotation of the cathode which results in a high degree of fiber alignment **(Fig.17).** Linear velocity of the rotating disk needs to be equal or higher than the fiber speed when it hits the cathode.

Conductive coverslips, is mounted on the surface of a rotating disk (**Fig.17. A).** The fiber alignment pattern, will be in the form of concentric circles. **(Fig.17. C & D)**

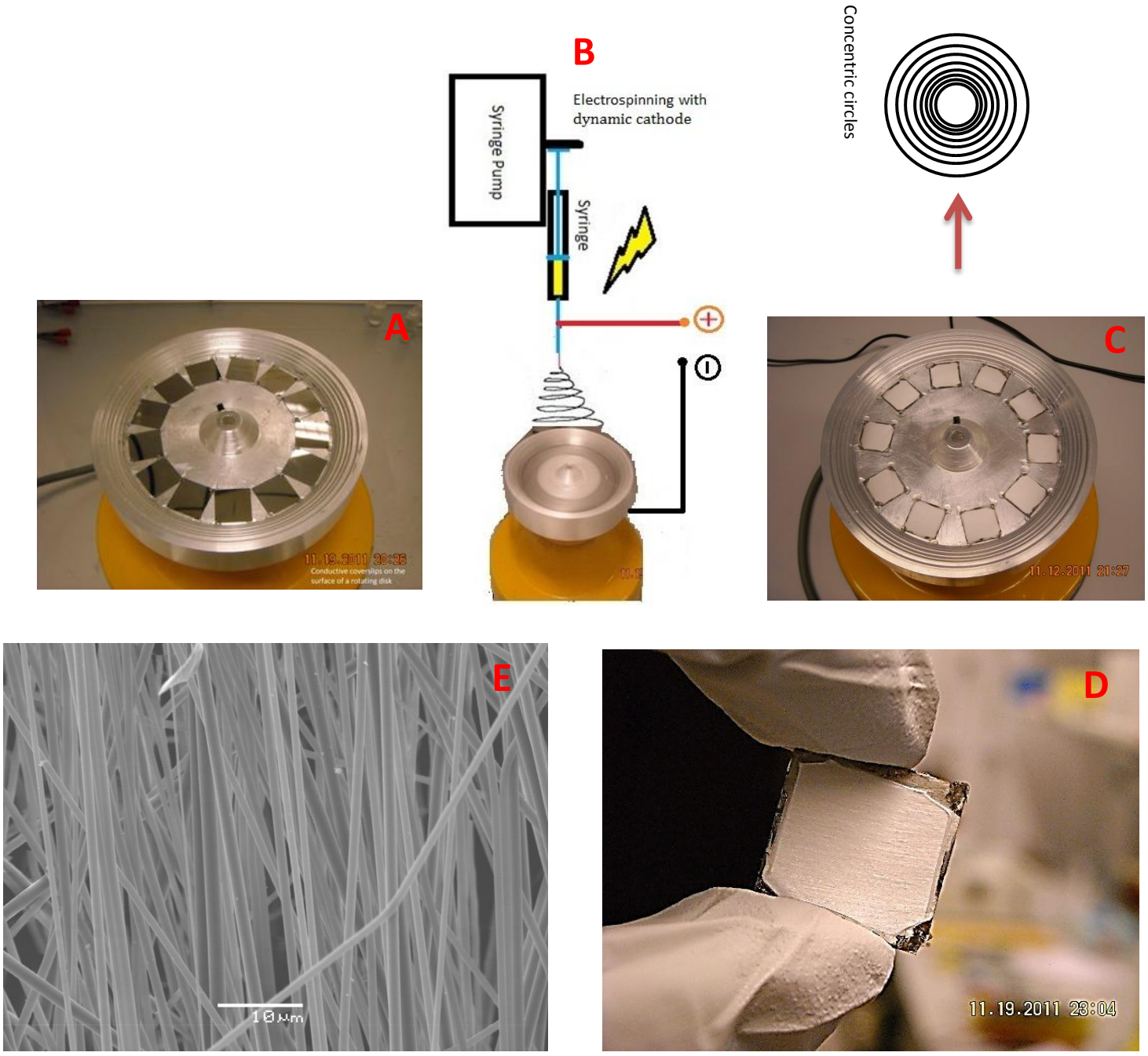


Fig.17. Electerospinning with Dynamic cathode

A) Conductive coverslips (Gold-Platinum coated), mounted on the surface of a 10 cm diameter Rotating disk.

B) Electerospinning setup

C & D) Concentric PLLA fibers accumulated on conductive coverslip/s

E) SEM image of the concentric PLLA fibers

If a thin plastic film band be wrapped around the edge of the same rotating disk, then highly aligned fibers can be formed on the surface of the plastic film **(Fig.18 - 22)**.

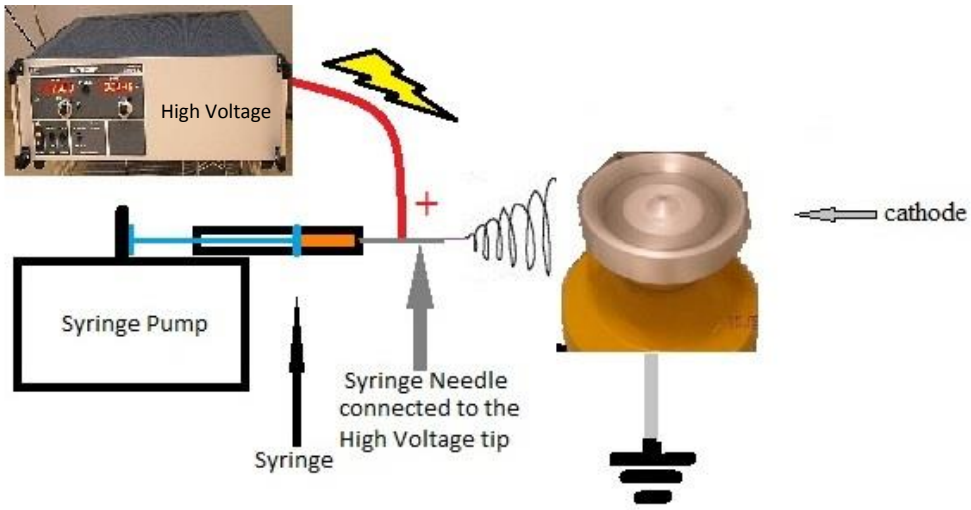


Fig.18. Electrospinning of highly aligned fibers on a thin plastic film band wrapped around the edge of a high speed rotating disk

Fig.19. Highly aligned electrospun PS fibers on a thin plastic film

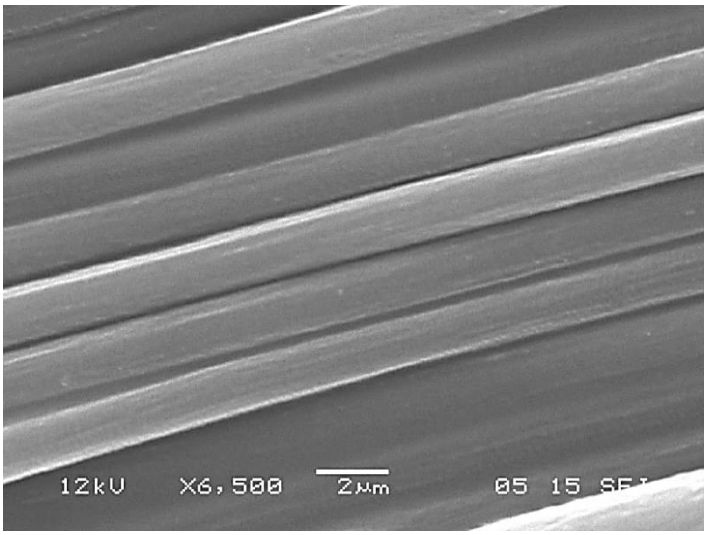


Fig.20. SEM image of highly aligned electrospun PS fibers on a thin plastic film

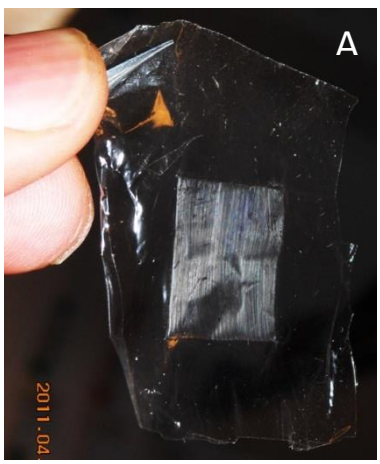


Fig.21. Highly aligned electrospun PLLA fibers on thin plastic films
A) Highly aligned PLLA fibers on 12.5 micron plastic wrap
B) Highly aligned PLLA fibers on 50 micron PLLA film

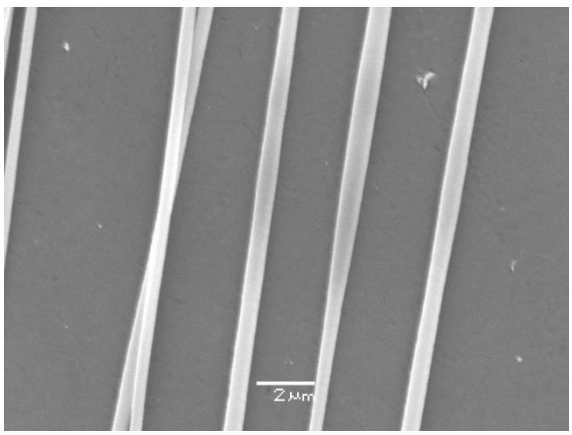


Fig.22. SEM image of highly aligned electrospun PLLA fibers on a 50 micron PLLA film

Converged & Diverged Fibers

In addition to concentric and parallel fibers, converged-diverged 2D & 3D fiber alignments were achieved and variety of samples was produced in this project. Converged fibers can be used to orient, guide and concentrate the random growth of an Axon population from one particular area towards a desired place in the body. Diverged fibers can be considered as a potential axon guide that may be able to guide axons from a densely packed area of axons to a wider area suitable for a neural network.

Custom and complex alignment

Depending on the application or the nature of the experiment, custom fiber alignment is possible. Different fiber types with different diameter, morphology and combination can combine and make complex structures for a specific application or experiment. This can be done by special design of the electrospinning system, anode or cathode and altering the spinning process and parameters. (e.g. see **Fig. 23)**

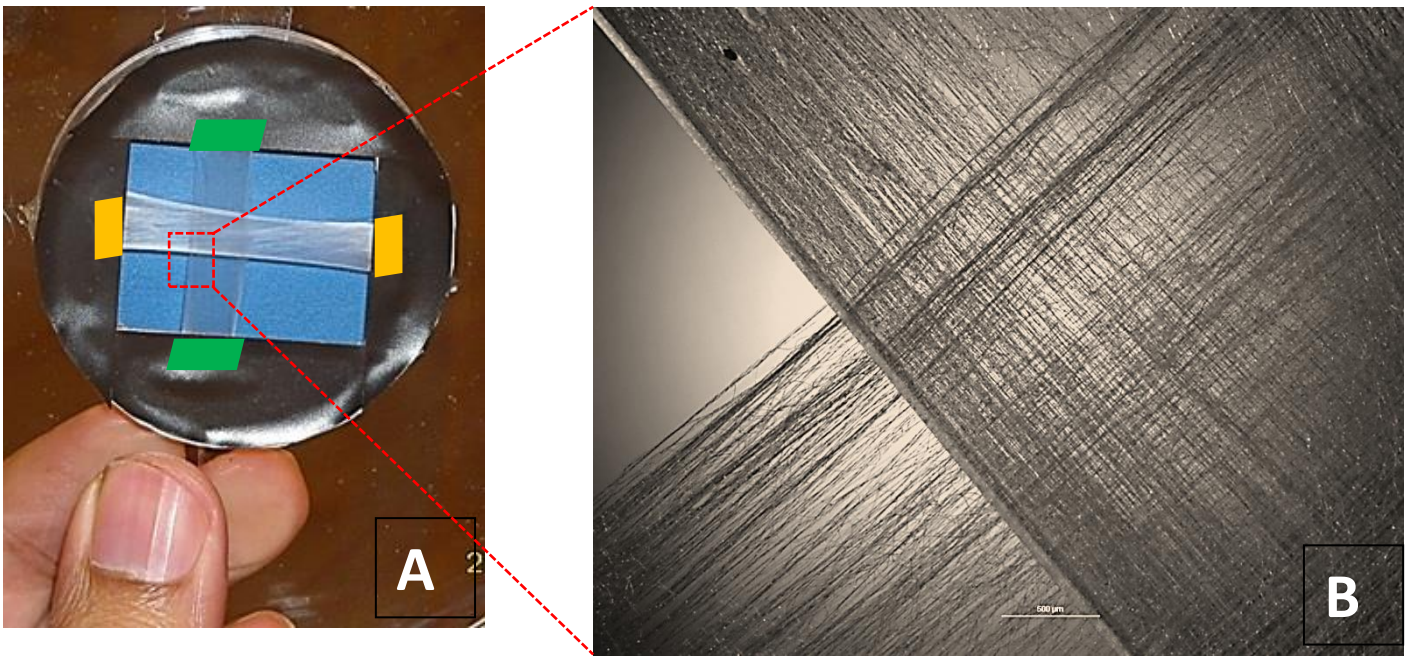
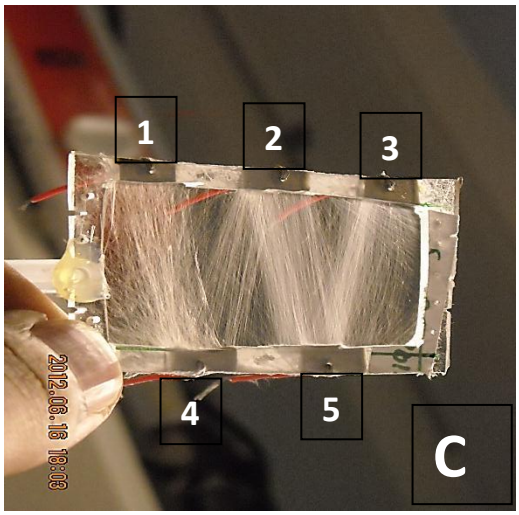


Fig. 23. Based on Gap Method, specially designed cathodes can create complex fiber alignments.
A, B) Two stripe patterns of EVA & PLLA parallel fibers perpendicular to each other on a specially designed of a 4 electrode cathode, first the yellow marked electrodes are used as a cathode and parallel EVA fibers are collected between the gap; and then the yellow electrodes are disconnected and the green marked electrodes are used as cathode and this time PLLA parallel fibers are collected between the gap.
C) Shows a 5 electrode cathode. By using a combination of these electrodes as a cathode, different patterns and fiber alignments can be achieved. In this example, the capital letter "W" has been created by PS fibers. The sequence of the used electrodes is : (1&4 → 4&2 → 2&5 → 5&3) the spinning time in each sequence in this example is 5 minutes.



By increasing the number of the electrodes on a 2d or 3d designed cathodes and using a combination of these electrodes as a cathode along with the use of multiplexing and switching techniques, complex 2D , 3D fiber structures for tissue engineering scaffolds, special medical implants and **filter industry** can be produced. Air filtering is essential for many industries and clinical applications. **High efficiency filtering with low pressure drop** is the main goal and nanofiber coating of the conventional filter media is a promising method on achieving this goal. However in order to achieve the optimal filtering performance, the spinning techniques need to be improved and I think this is possible.

Nanofiber Yarn

Fibers can be produced in the form of yarn. In this case every thread may consist of thousands of continues and aligned fibers. There are several "Nanofiber Yarn" production methods, however water based method is shown in here. **(Fig. 24-25).** Fibers can be simple component or composite. Advanced combinations are also possible. Nanofiber yarn has potential applications in textile and filtering industry, tissue engineering, advanced sensing as well as in biophotonics and neuroscience. For further information, the interested reader is referred to **Ref. [34, 35]**

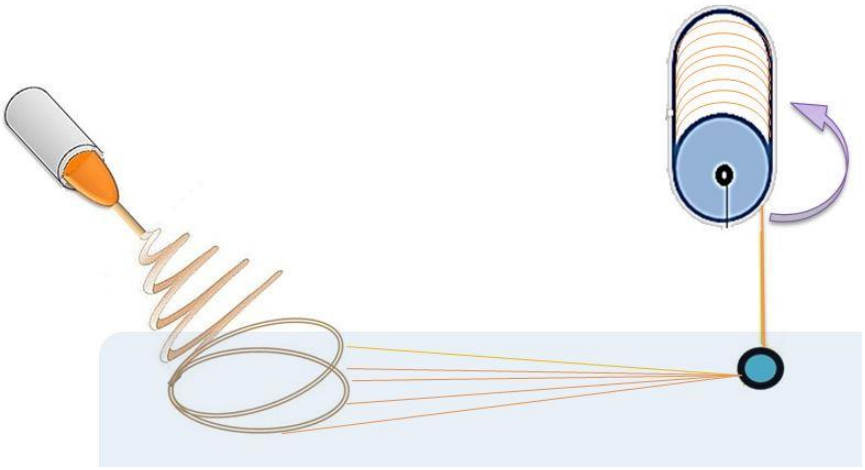


Fig.24. Fibers are spinning randomly on the surface of water while being pulled by a small pulley on the surface of the water. Fibers become aligned during their movement towards the small pulley. Aligned fibers are collected by a Take up Wheel. (Technical details are not shown)

Fig.25. PAN (polyacrylonitrile) nano yarn. Every thread consists of thousands of continuous and aligned nanofibers collected by a take up wheel. Ref .[35]

Chapter 3 (*Composite fibers, Coaxial electrospinning - Metallic nanotubes*)

Different Composite fiber production methods and hollow, magnetic and metallic fiber production techniques that were used in this master work are briefly discussed in this chapter.

Composite fibers

Fibers can be a mixture of several different materials including polymers, nanomaterial or biological entities and agents (e.g. live cells, drugs, antibodies,…).
Composite fibers are produced through the electrospinning technique in several different ways. Some of these methods are shown in this report:

- *Single needle injection I* (*e.g.*** PLLA + BSA protein composite fibers)**

Solid or liquid additives are mixed with the polymer solution, stirred and then spun through a single needle injection. PLLA solution was mixed with a bovine serum albumin (BSA) protein dissolved in PBS (phosphate-buffered saline) buffer. The mixture was stirred for 2 hours and then spun using a single needle spinning process (**Table.1**)

Experimental condition:

Additive	Polymer	Solvent	Concentration	Used Polymer solution (ml)	Flow rate (ml/h)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Spinning Time (min)	Fiber diameter (µm)
0.01gr BSA protein dissolved in 0.1ml PBS	Poly-L-Lactide Acid	Chloroform	15 % W/W	1	0.8	22	0.4	20	72	≈ 3

Table.1

Single needle injection II (*e.g.* Polystyrene + Polyimide composite fibers)
Solid or liquid additives are mixed with the polymer solution in injection needle just prior to injection.
Two syringe pumps were used and two polymer solutions were mixed in injection needle just prior to injection in the spinning process (**Fig. 27-28**).

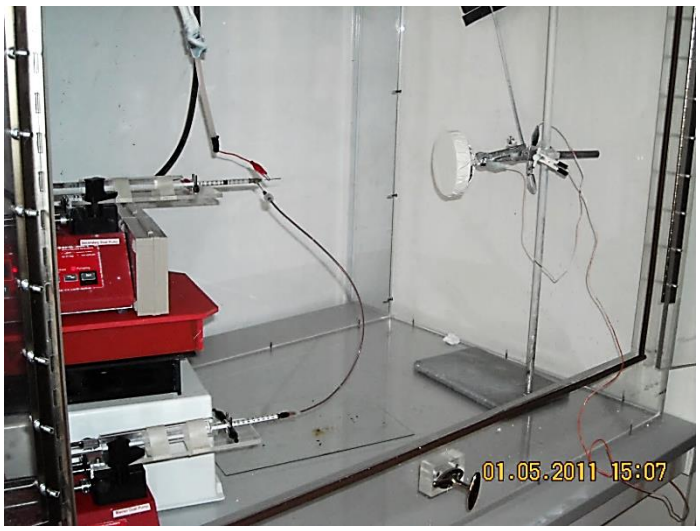


Fig.27. Polystyrene-Polyimide composite fiber spinning

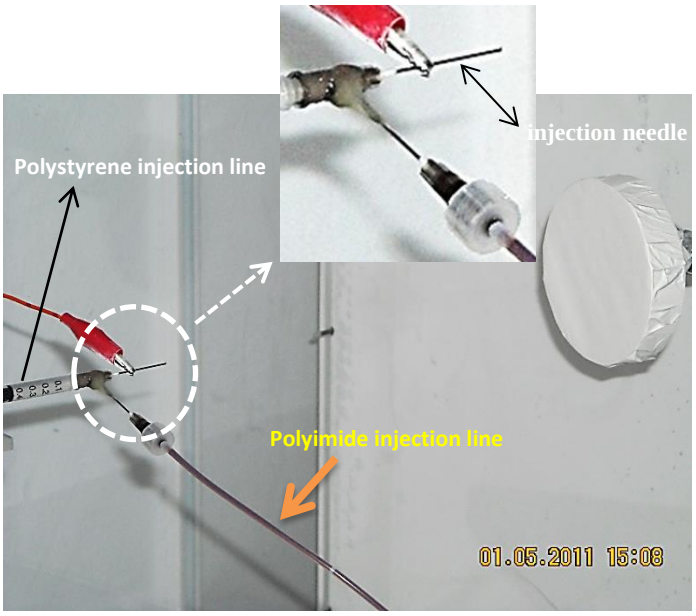


Fig.28. Mixing two polymer solutions in injection needle

Experimental condition:

Polymer 1	Polymer 2	Solvent	Polymer Concentration (is the same for both polymers)	Flow rate (ml/h) (is the same for both polymers)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Spinning Time (min)	Fiber diameter (μm)
PS (Polystyrene)	Polyimide	DMF (dimethylformamide)	15 % W/W	0.6	30	0.4	20	20	≈ 0.3

Coaxial electrospinning

In coaxial electrospinning, a specially designed coaxial nozzle, consisting of two concentric orifices is used (**Fig. 29 A**). The nozzle was specially designed. A simple way of making a coaxial nozzle is to use two syringe needles that are connected together by a pipet tip and fixed by applying plastic glue (**Fig. 29 B**). Each needle can provide the pipet tip with different additives and solutions. The middle needle is connected to the high voltage tip. Solid or liquid additives are added to (or encapsulated in) the polymer solution through a core or shell orifice of a coaxial nozzle (Fig.29). Two syringe pumps are used. Samples of micro-nano fibers/tubes spun using coaxial electrospinning are shown in the following pages (*e.g.* Hollow fibers see **fig.30** , Polystyrene + Polyimide composite fibers see **fig.31** , Magnetic fibers see **fig.33-34**).

Using a coaxial electrospinning, nanoparticles, proteins, liquid droplets, drugs and fluorescent dyes or molecules can be embedded in or attached to the surface of the fibers or the beads. Some biological specimens like DNA, viruses, bacteria or living cells can be encapsulated in the fibers or beads. Fibers can be a combination of two or more polymer layers. They can be hollow, conductive, have diverse morphology, have magnetic properties or guide light. The additives or entities and agents (*e.g.* live cells, antibodies) can inject through the core orifice while the protective polymer solution can inject through the shell orifices. However this can change regarding to different applications.

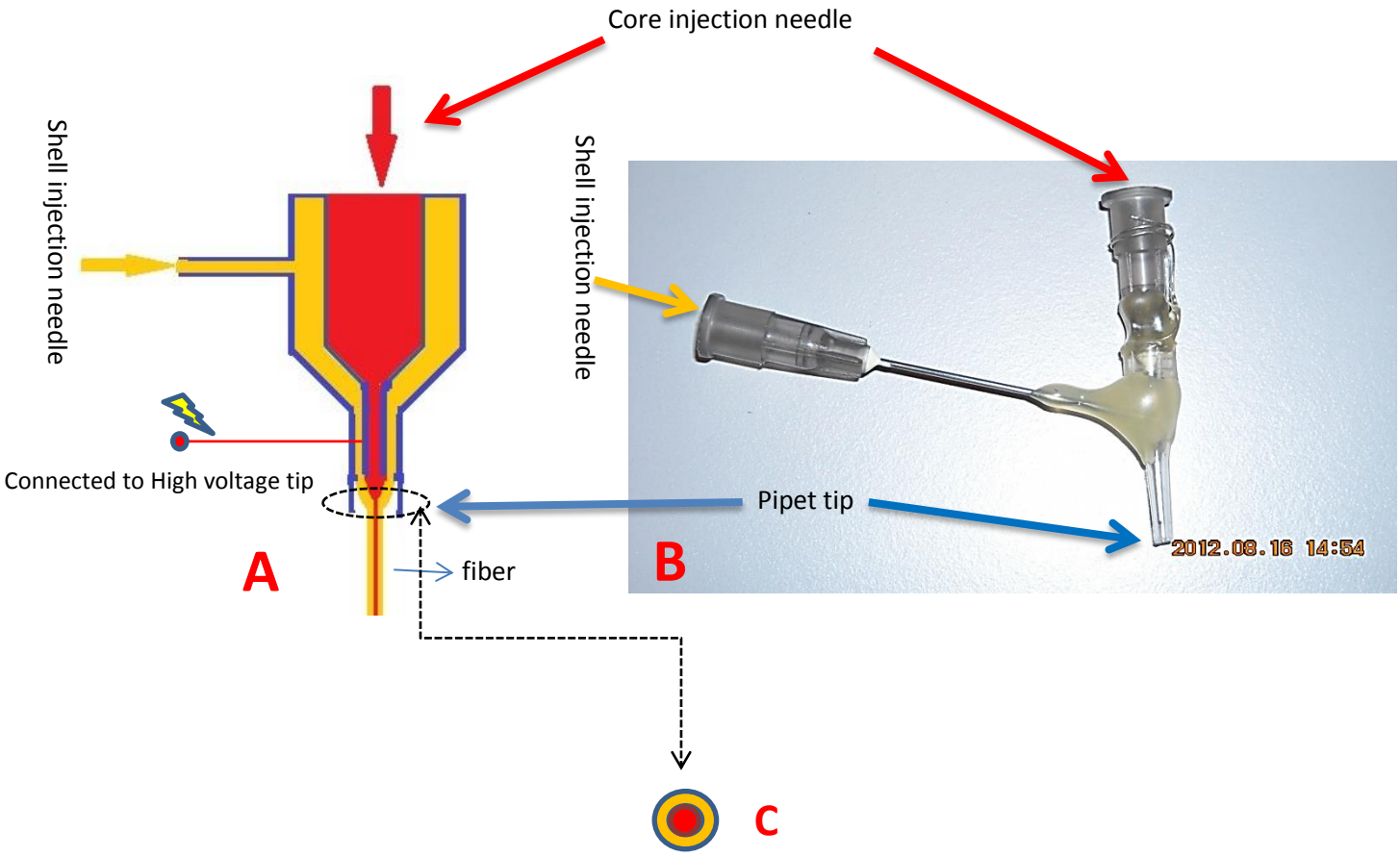


Fig.29. Coaxial electrospinning

A) A simple diagram of a coaxial nozzle.

B) Actual nozzle.

C) Cross sectional view of the concentric orifices

Hollow fibers (Oil + PLLA solutions)

Hollow fibers are formed when the core needle injects liquids like oil (cooking oil in here) and the other needle injects polymer solution. The liquid evaporates after spinning, leaving hollow fibers behind. (**Fig. 30**)

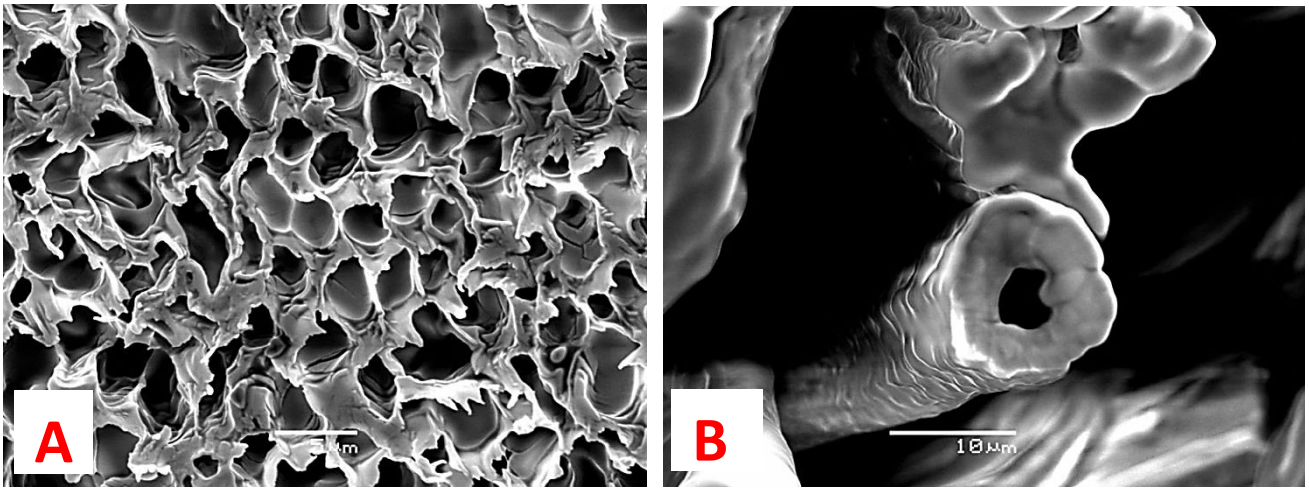


Fig.30. Hollow fibers

A) SEM image of a cross section of an assembly of aligned PLLA hollow fibers with thin shell (Gap Method is used.)
B) SEM image of a single PLLA fiber with thick shell and nanostructure surface

Experimental condition:

Type	Polymer	Solvent	Concentration	Core Flow rate (ml/h)	Shell Flow rate (ml/h)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Spinning Time (min)	Hollow Fiber diameter (µm)
A	Poly-L-Lactide Acid	Chloroform	15% W/W	0.2	0.5	22	0.4	20	20	≈ 2
B	Poly-L-Lactide Acid	Chloroform	15% W/W	0.2	0.9	22	0.4	20	20	≈ 10

Polystyrene + Polyimide composite fibers

Two syringe pumps were used and two polymer solutions were joined together at the tip of a coaxial nozzle and formed two-layer composite fiber in the spinning process (**Fig. 31-32**).

Fig.31. Polystyrene + Polyimide composite fibers spun on aluminum foil

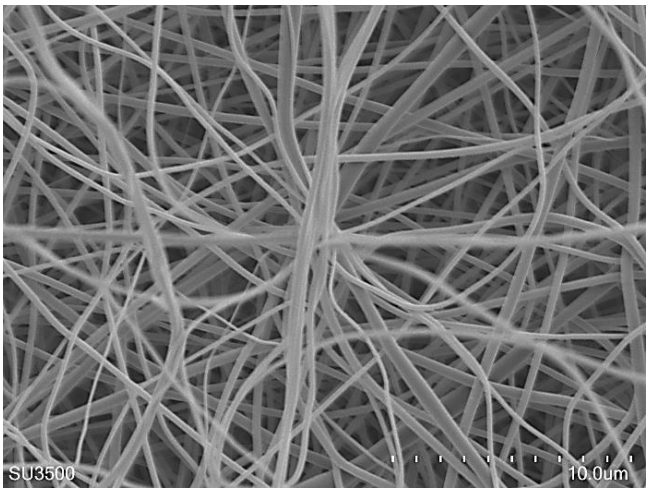


Fig.32. SEM image of Polystyrene + Polyimide composite fibers

Experimental condition:

Core Polymer	Shell Polymer	Solvent	Polymer Concentration	Core Flow rate (ml/h)	Shell Flow rate (ml/h)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Spinning Time (min)	Fiber diameter (µm)
PS (Polystyrene)	Polyimide	DMF	15% W/W	0.3	0.3	30	0.4	20	20	≈ 0.3

Magnetic fibers (Iron oxide nanoparticle + EVA solution)

Adding magnetic nanoparticles to the polymer solution gives the electrospun fibers magnetic properties. This gives us the ability to manipulate the fibers using electromagnetic fields. (**Fig. 33-34**)

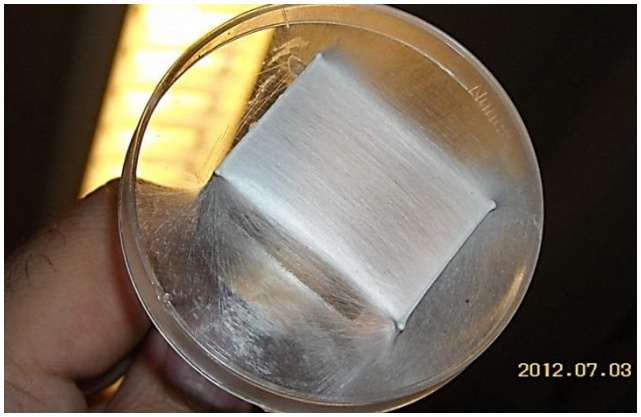


Fig.33. Aligned "EVA+Iron oxide nanoparticle" composite fibers. Gap Method and coaxial spinning is used. Iron oxide nanoparticles mixed with EVA polymer solution and injected from core orifice of the nozzle while shell orifice only injected EVA polymer solution.

Fig.34. "PS+Iron oxide nanoparticle" composite fibers attracted by a magnet

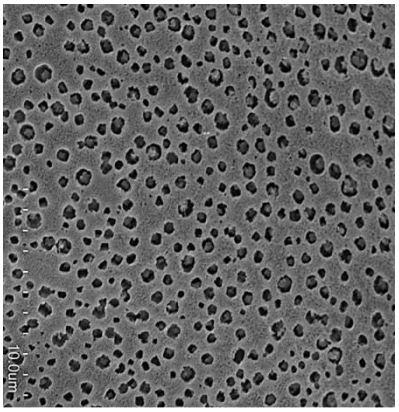


Fig.35. SEM image of iron oxide nanoparticles Produced through an electrolysis process.

Experimental condition:

Additive	Polymer	Solvent	Polymer Concentration	Core Flow rate (ml/h)	Shell Flow rate (ml/h)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Spinning Time (min)	Fiber diameter (µm)
Iron oxide nanoparticle 0.1gr/1ml polymer solution	EVA (ethylene vinyl acetate)	Chloroform / DMF (7/3) (V/V)	10 % (W/W)	0.3	0.3	35	0.4	20	20	≈ 2

Metallic nanotubes

In this study electrospun aligned fibers are used as a template for metal nanotube production. In this experiment first aligned polystyrene nanofibers were produced through the GAP method. Sputtering technique was used and fibers were coated with a thin layer of metal (gold-palladium alloy). Then the coated fibers were baked in the oven for 10 minutes at 280 ˚C. When the temperature reaches to 240 ˚C, the polystyrene starts to melt and by increasing the temperature over 240 ˚C, polymer starts to evaporate and in the end the only remnants are metallic tubes (**Fig.37**). See a 3D image in Appendix 3. **(Fig.73)**

There is a possibility to fuse all the sputtered metal particles firmly together by heating the metallic shells near to their melting point in an inert environment. This may result in smooth and strong metallic nanotubes. It may be possible to produce these tubes continuously with unlimited length. Their cross sectional shape can also be altered. Fusing the metal particles together using heat has already been done on electrospun metal nanofibers **Ref. [36].**

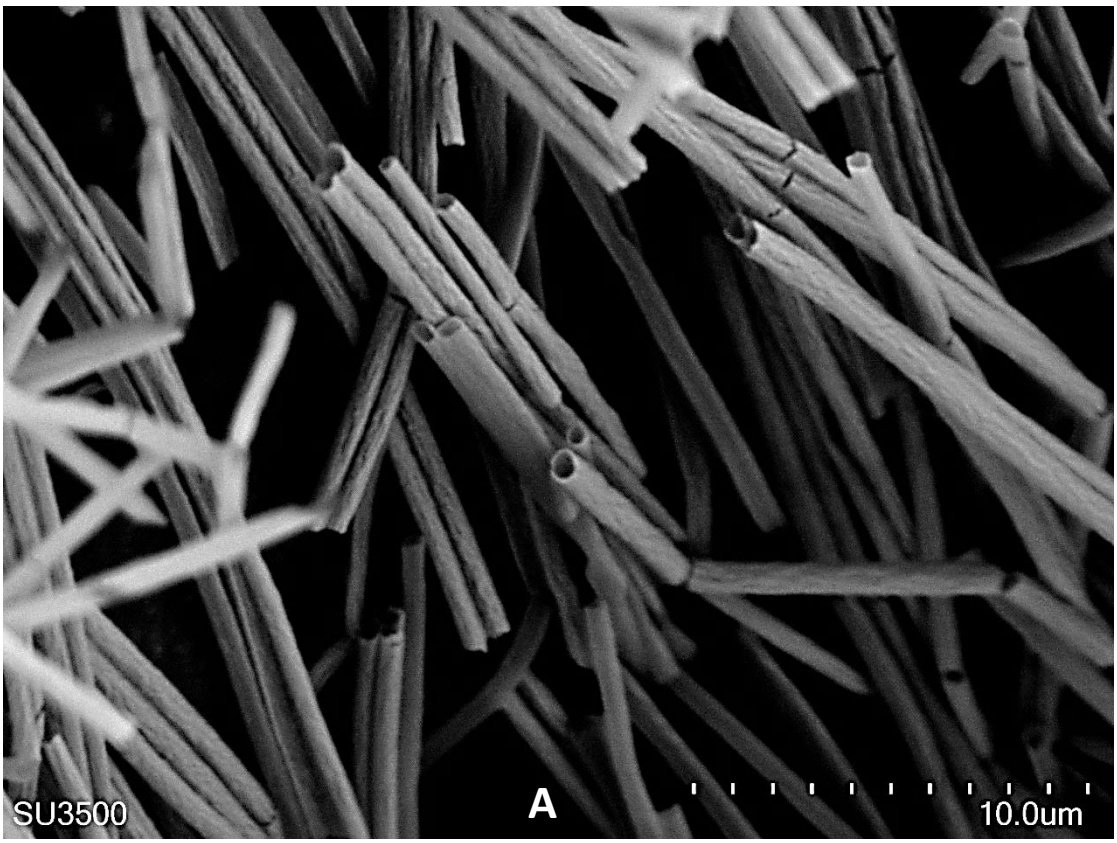


Fig.37A. Gold-palladium nanotubes produced through electrospinning during this master work

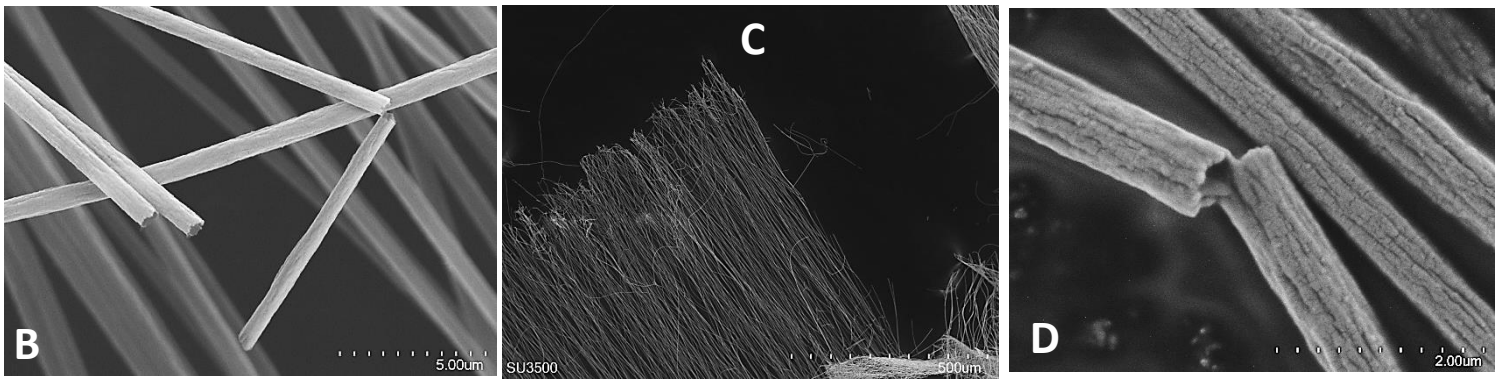


Fig.37. B, C, D Aligned gold-palladium nanotubes produced during this work

Skin effect in metals: In higher frequencies, the electric current is forced to flow mainly through a thin cross-sectional area near the conductor surface. This phenomenon is known as skin effect. In a cylindrical conductor (**Fig.38 A**), approximately 63% of the total current, flows through this area. The width of this area is known as the skin depth or Δ**.** (Fig.38A. Yellow ring area).

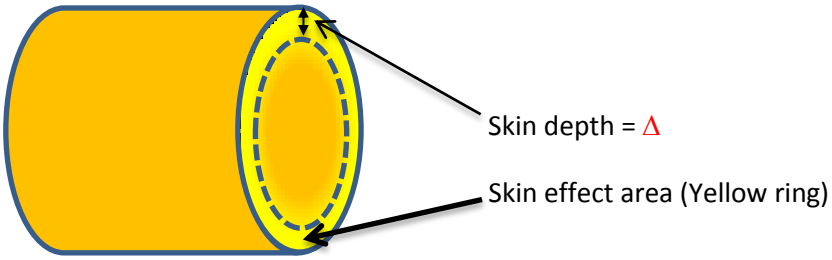


Fig.38 A. Cylindrical conductor

Skin depth depends on the current frequency and the electromagnetic properties of the conductor. (See Eq.1)

$$\text{Eq.1} \qquad \Delta = \sqrt{\frac{\rho}{\mu \pi f}}$$

where ρ is the conductor resistivity, f is the current frequency and μ is the metal permeability **Ref [23].**
The equation clearly indicates that for a constant permeability and in a specific frequency, Δ remains constant. In case of a cylindrical conductor this means that most of the current flows through the Skin effect area (Yellow ring area).

Comparison!

I like to imagine a microwire with cross-sectional area of **A,** suddenly changes to an assembly of insulated nanowires (or nanotubes) within the same cross-sectional area. **(Fig.38 B).** In one specific frequency, the total skin effect area of the nanowire/tube assembly will be much larger than the skin effect area of the single microwire. This indicates that in a specific high frequency, an assembly of metal nanowires/tubes with cross-sectional area of **A** is capable of carrying much more current in comparison with a single microwire with the same cross-sectional area.

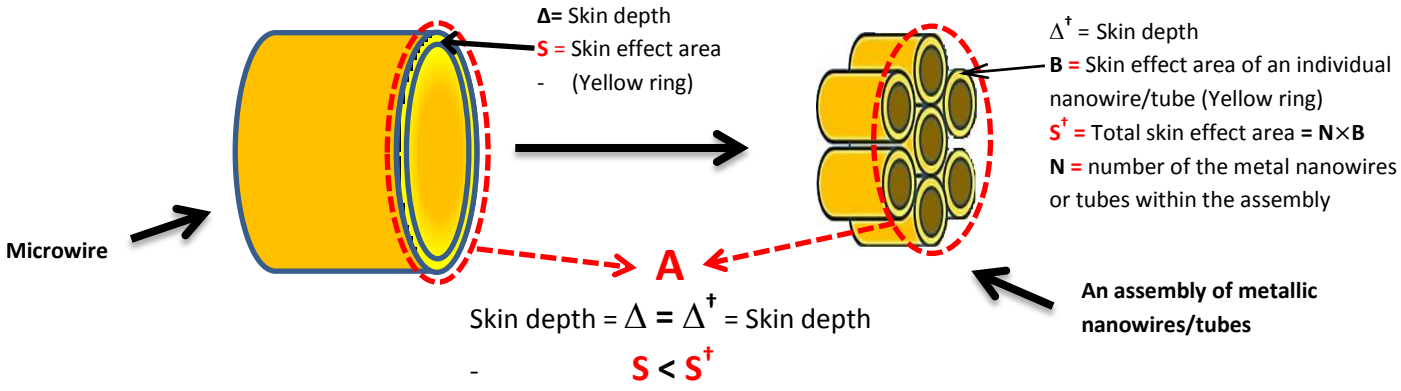


Fig.38 B. Comparison on Skin effect area between a microwire and its equivalent nanowire/tube assembly

Applications: Metallic nanotubes have the potential to be used as drug or gene delivery or protein transport systems or used in ultra-small sensitive nanodevices like sensors in nanoeletronics **Ref [12, 13]** and in biochemical devices as biosensors like enzyme sensing devices **Ref [14].**
Nanowires may have the potential to stimulate and record the activity of large numbers of neurons with single-cell resolution **Ref. [37].**

Metallic long nanotubes also may have the potential to be used as neural electrodes. Foreign body reaction decreases when the size (diameter) of the implanted electrode decreases. When the electrode becomes thinner, it becomes more flexible and can follow the brain tissue movements that can lead to a flexible anchoring and less tissue inflammation. An assembly of thousands of conductive long nanotubes may be able to be used as "Brain-Machine" mass data transfer interface electrodes.

Chapter 4 (*Tissue Engineering*)

Tissue Engineering & Scaffolding

"Nonstructural characteristics" of ultra-thin fibers can mimic the extracellular matrix of the native tissue; therefore these fibers are considered as very promising candidates for making scaffolds for tissue engineering. Extracellular matrix (ECM) consists of fibrous proteins in a 3D structural network suspended in a GAG (glycosaminoglycan) hydrogel. In addition to their morphology, high porosity and high aspect ratio along with their mechanical strength make the synthetic fibers more attractive in tissue engineering. However these polymers suffer from lack of cell recognition signals while natural materials suffer from poor mechanical strength when they come in contact with water. Mixing natural and synthetic materials or covering (immobilization) the surface of the synthetic fibers with natural materials can improve both mechanical strength and biocompatibility of the resultant fibers **Ref [15].**
Two sample scaffolds for bone and blood vessels that were produced during this master work are shown in this report. (**Fig.40-41**).The sample for the bone scaffold may suggest a hollow fiber based method for bone regeneration.

Scaffolds for connective tissue regeneration I (Skin),
Biodegradable polymers like PLLA (poly L-lactide Acid), PGA (poly glycolide) and PCL (poly caprolactone) are used in variety of studies for applications in skin, bone and blood vessel scaffolds **Ref [15].**
Sheets of aligned and random fibers from different polymers were produced during this work. Sheets of random fibers were produced through spinning on a stationery cathode like a metal plate or aluminum foil while sheets of aligned fibers were produced through spinning on a dynamic cathode like a high speed rotating disk. Thin sheet scaffolds are used in skin regeneration studies. These sheets can take the shape of different molds. 3D scaffolds also can be made by spinning on a stationary or a dynamic mold where the mold itself acts as a cathode. Image 39A show an example of a cone like shape sample scaffold; produced during this project. (**Fig. 39 A, B**)
More complex structures are also possible.

Fig.39 A. Sample 3D scaffold of concentric PLLA fibers
- produced by spinning on a rotating mold

Fig.39 B. Rotating mold

Scaffolds for connective tissue regeneration II (Bone - Connective tissue)

Some studies show that 3D porous structures made of "Collagen + carbon nanotubes (CNTs)" and "poly-L-lactic acid (PLA) + carbon nanotubes (CNTs)" have the potential to be used as a Bone Regeneration Scaffolds. Addition of CNTs improves the mechanical properties of the scaffolds. CNTs also can be functionalized with biofunctional groups like growth factors or cellular ligands that can affect signaling and cell key functions. In addition to the biodegradability, bioresorbablity and rigidity, a potential bone regeneration scaffold needs to have a highly 3D porous structure with Nano structural surface. Porosity can maximize the integration of the body fluids with cells while nanostructured surface can enhance the cell adhesion **Ref. [16].**

In this study, L929 Cell Line from mouse was cultured on glass coverslips coated with electrospun PLLA random and aligned fibers (Fig. 40A). Culture medium was added and the samples were incubated for 3 days. Then the samples were prepared for the SEM observation. Scanning Electron Microscopy Images show that the cells nicely are attached to the fibers and the cell-division cycle clearly can be realized (See Fig.40B & C).

Some of the cells could penetrate and attach themselves to the fibers beneath the Surface and in deeper layers of the scaffolds and experience a true 3D environment (Fig. 40D).

This observation supports the possibility for making 3D fiber based scaffolds for bone and connective tissue regeneration.

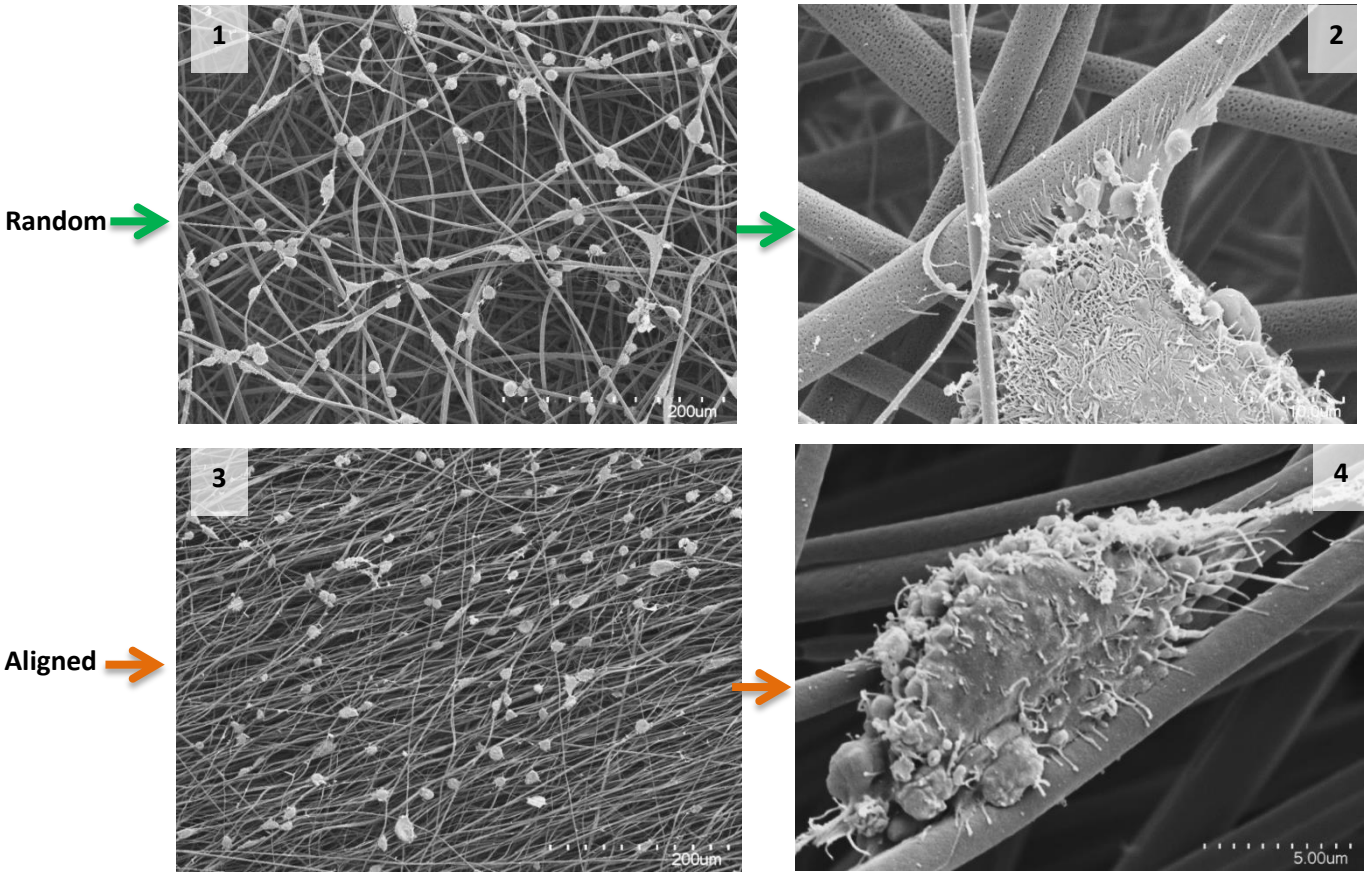
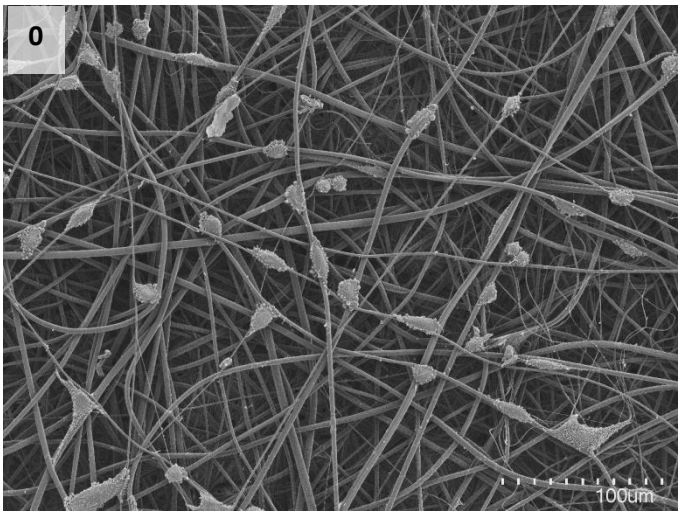


Fig.40A. 3D fiber based scaffolds

1. Fibroblasts cultured on PLLA random fibers 2. A close view of a fibroblast-PLLA random fiber attachment, fiber porosity enhances the cell-fiber attachment.
3. Fibroblasts cultured on PLLA aligned fibers 4. A close view of a fibroblast-PLLA aligned fiber attachment

Experimental Conditions:

Type	Polymer	Solvent	Concentration	Flow rate (ml/h)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Cathode Rotation speed (RPM)	Spinning Time (min)	Fiber diameter (μm)
Random	Poly-L-Lactide Acid	Chloroform	15% (W/W)	0.8	22	0.4	20	0	40	≈ 3
Aligned	Poly-L-Lactide Acid	Chloroform	15% (W/W)	0.8	22	0.4	20	7000	20	≈ 2



Cell-division cycle on random PLLA fibers

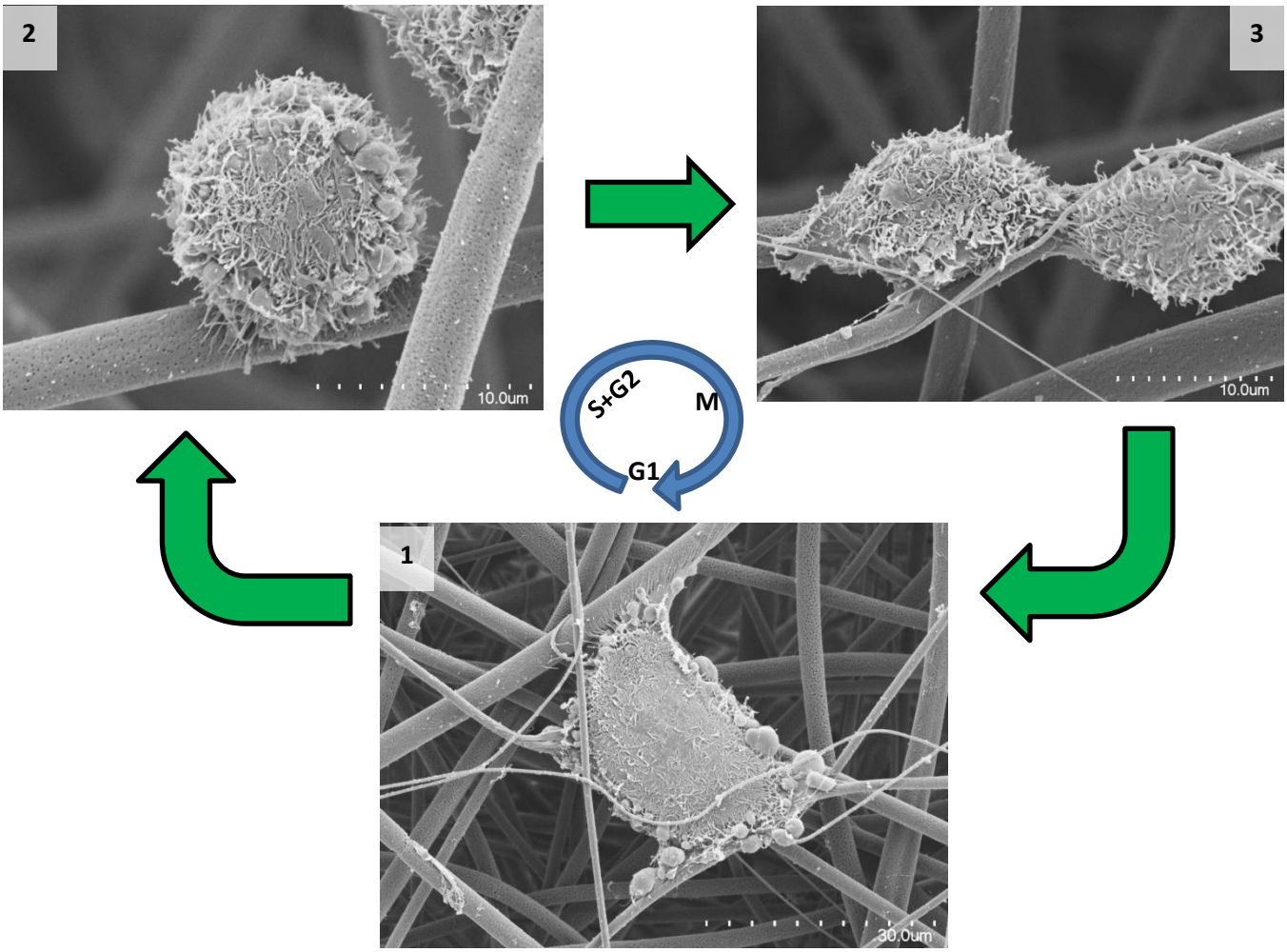
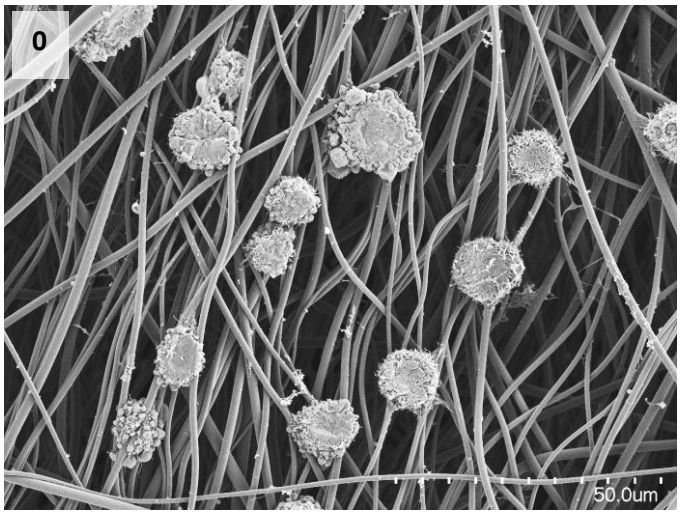


Fig.40B. Cell-division cycle on random PLLA fibers

0- Fibroblasts on random PLLA fibers with cells in different cell cycle process
1- A mutual Cell attached to the fibers, cell grows (G1)
2- Pre-division state, DNA replicates, chromosomes duplicate, cell grows and prepares itself for mitosis (S+G2)
3- Nucleus divides and Cell-division occurs (Mitosis)



Cell-division cycle on aligned PLLA fibers

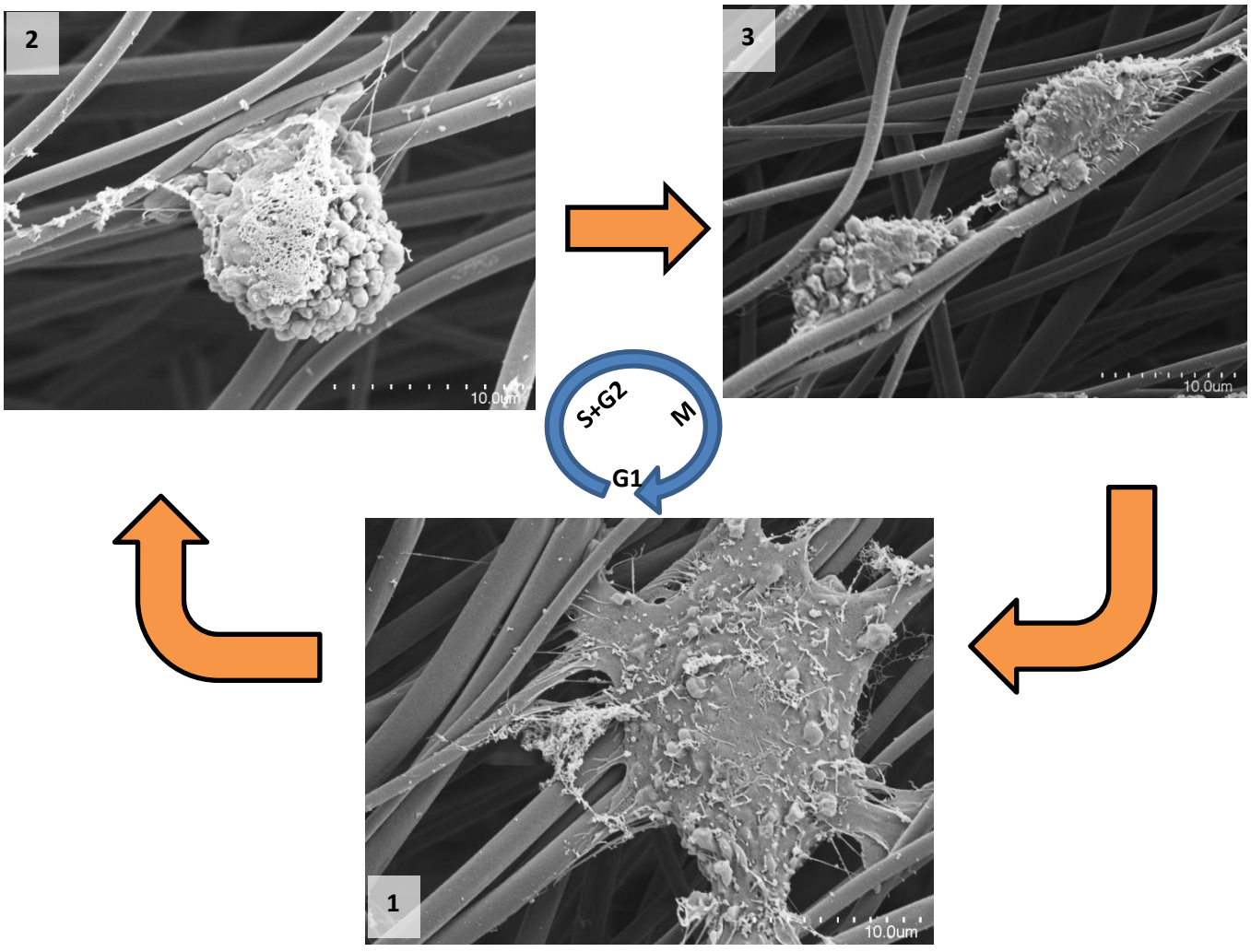


Fig.40C. Cell-division cycle on aligned PLLA fibers

0- Fibroblasts on aligned PLLA fibers with cells in different cell cycle process
1- A mutual Cell attached to the fibers, cell grows (G1)
2- Pre-division state, DNA replicates, chromosomes duplicate, cell grows and prepares itself for mitosis (S+G2)
3- Nucleus divides and Cell-division occurs (Mitosis)

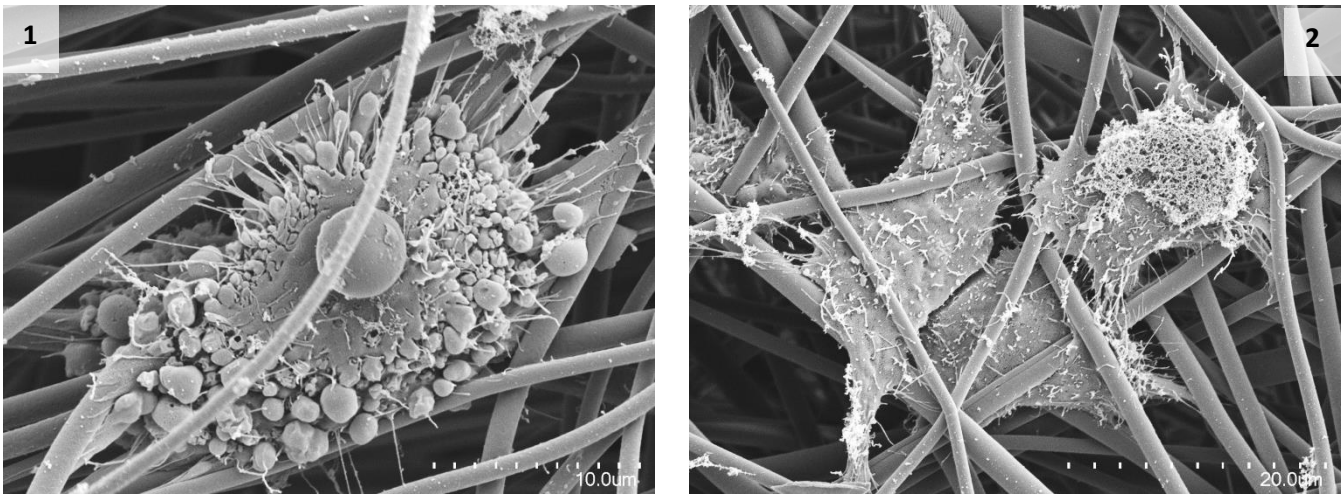


Fig.40D. Fibroblasts trapped in 3D structure of PLLA fibers.

1. Trapped cells in 3D structure of PLLA aligned fibers. The image shows a cell-division within the fibers. For a better view please see the corresponding 3D image (Appendix 3).
2. Trapped cells in 3D structure of PLLA random fibers. (See also a related 3D image in Appendix 3)

High porosity 3D structures:

In coaxial electrospinning if the viscosity of the core material is low, then the core jet will break-up easily. This will lead to the formation of beads. In case of oil or liquid core, the liquid can be encapsulated in the beads along the fibers **Ref. [17].**

The oil or liquid may also be able to escape from the fibers, leaving large pores on the surface of the fibers.

Using Gap method and coaxial electrospinning an assembly of high porosity hollow fibers were developed. (**Fig.40E**) SEM images of the cross sectional and side sectional view of the surface of this assembly are shown in **Fig.40F**

This approach may suggest a new method on making 3D porous structures for bone and connective tissue regeneration.

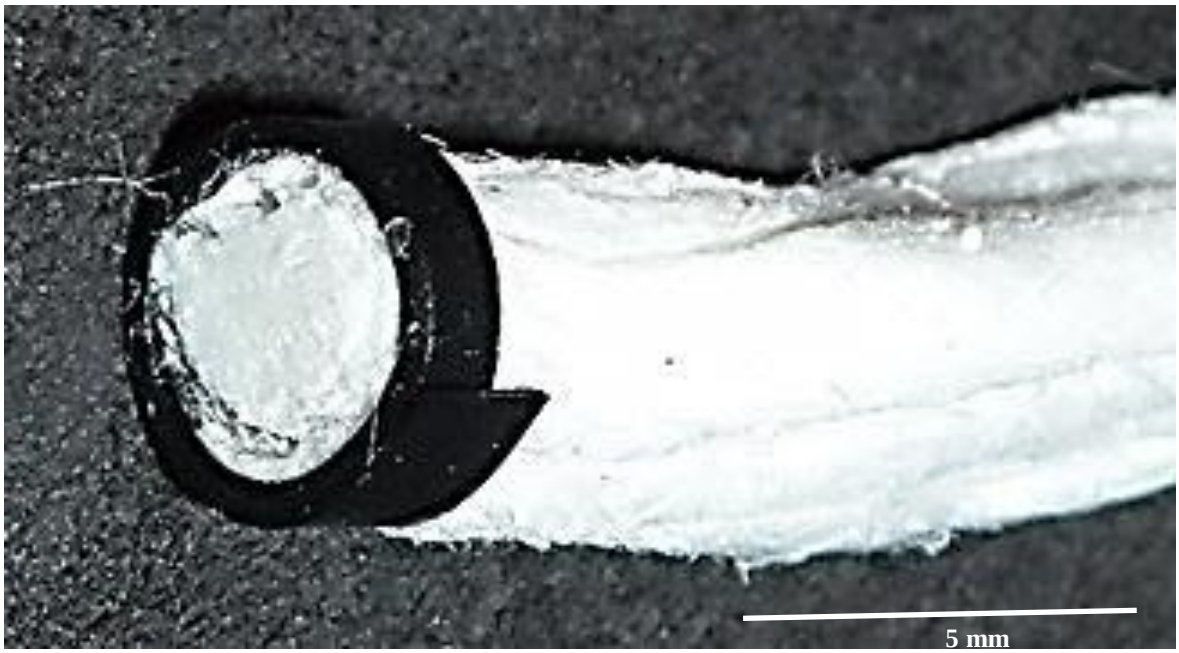


Fig.40E. 3D structure of high porosity aligned PLLA hollow fibers was developed during this master work.

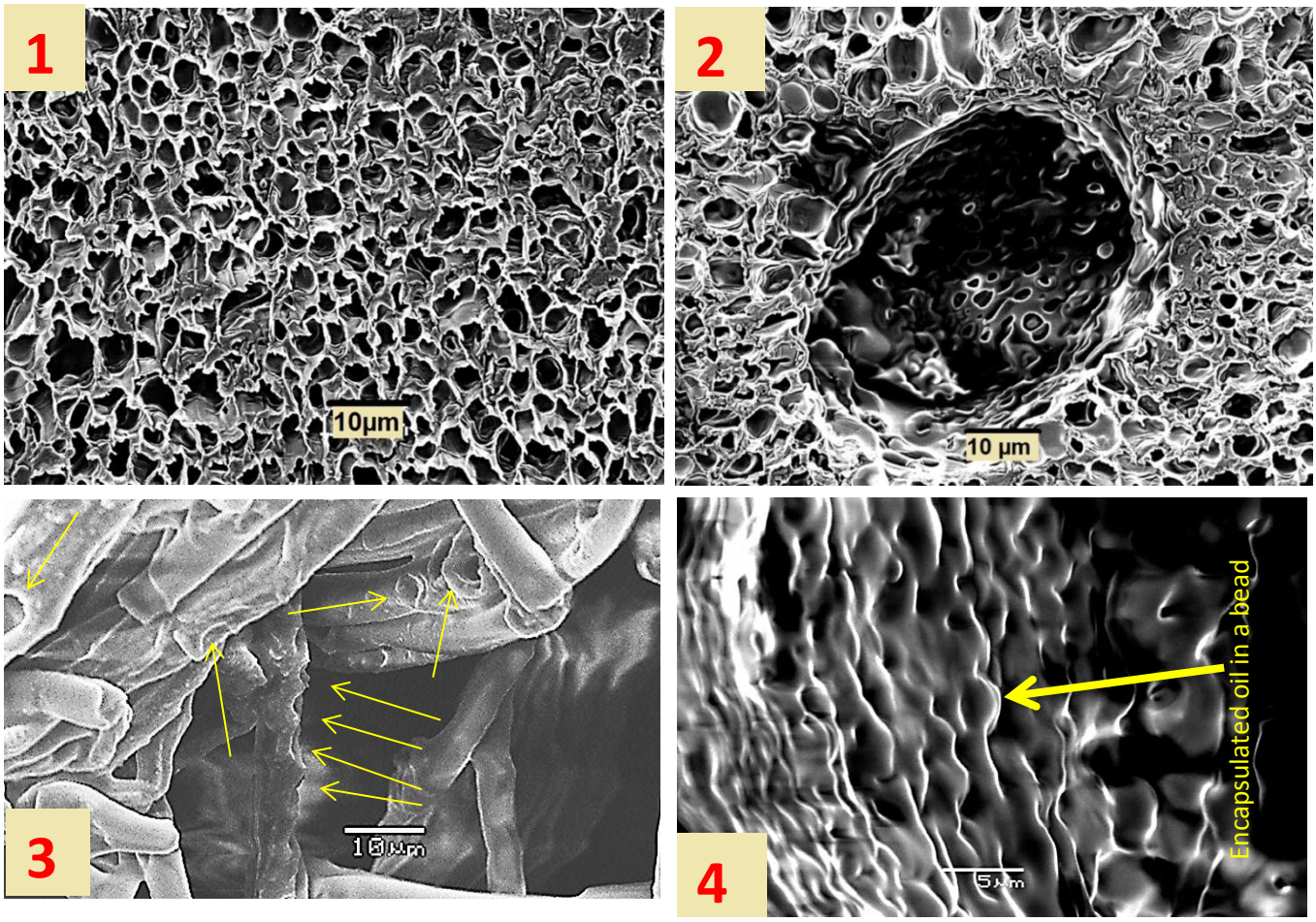


Fig.40F. SEM images of a sample PLLA hollow fiber based 3D porous scaffold.

1) Cross sectional view of an assembly of high porosity aligned PLLA hollow fibers.
2) Shows the scaffold with a damaged site. It is possible to see the interior porous structure through the damaged area.
3) Shows the large pores on the surface of the PLLA hollow fibers (Yellow arrows). These pores are formed when the oil (cooking oil in here) escapes the fibers.
4) Shows encapsulated oil in the beads along the fibers. If during spinning process the beads burst out then the oil can escape from the fibers leaving large pores behind.

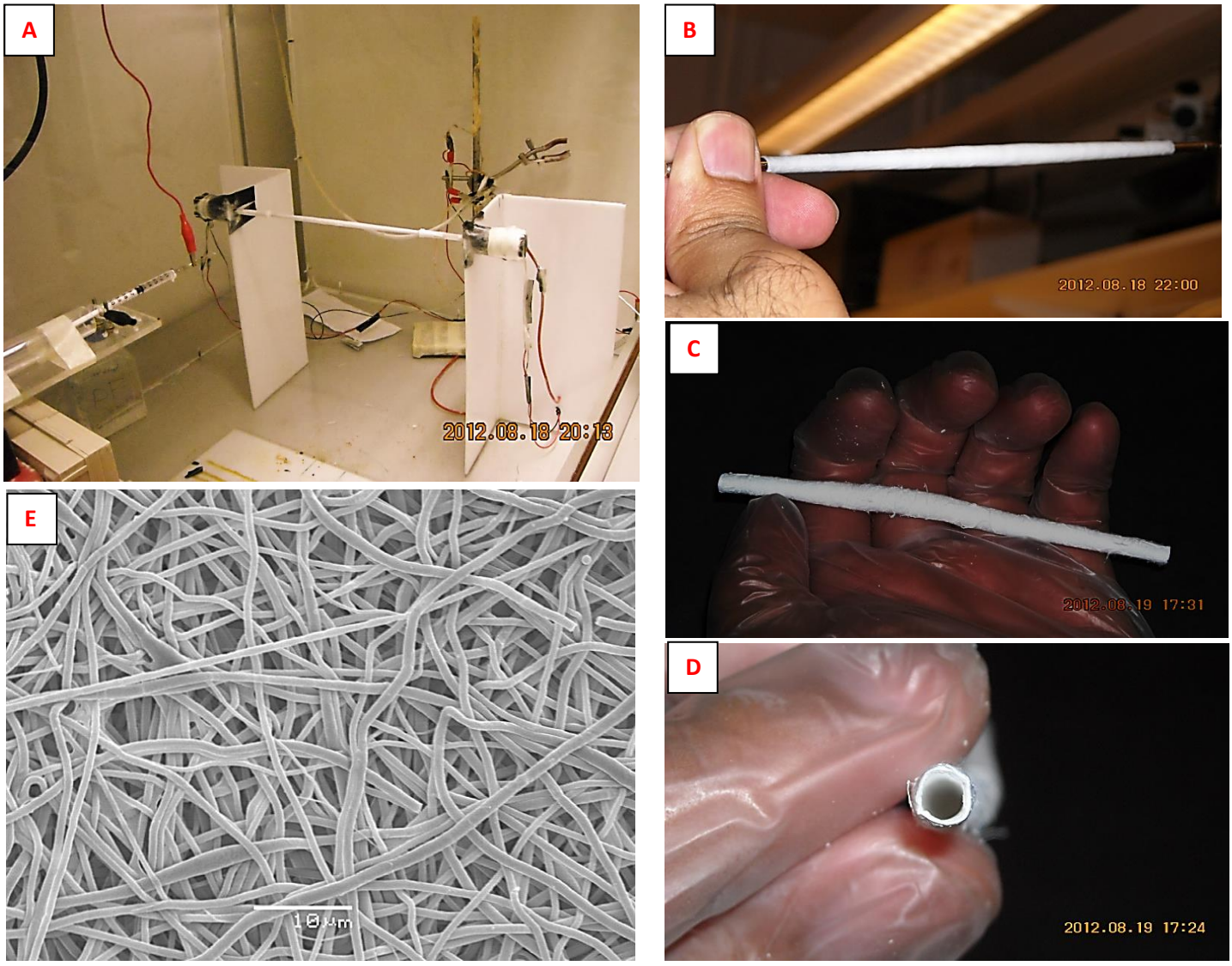
Experimental condition:

Core	Shell Polymer	Solvent	Concentration	Core Flow rate (ml/h)	Shell Flow rate (ml/h)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Spinning Time (min)	Hollow Fiber diameter (µm)
Cooking oil	Poly-L-Lactide Acid	Chloroform	15% (W/W)	0.2	0.4	22	0.4	20	20	≈ 3

Scaffolds for connective tissue regeneration III (Blood vessel**)**

Previous studies show that collagen-blended P(LLA-CL) nanofibers with diameter around 100-200nm have potential applications on vascular scaffolds **Ref. [15].**

Regardless of the polymer(s) that may be used in the scaffold, the following technique is used for the production of a sample blood vessel scaffold through the electrospinning technique.
A rotating metal mandrel is used as a cathode (**Fig. 41 A**). The PLLA fibers were deposited on the surface of the mandrel and formed a cylindrical thin mat on the surface of the mandrel (**Fig. 41 B**). The resultant mat (scaffold) then was pulled out of the mandrel (**Fig. 41 C**).



- **Fig.41**. Blood vessel scaffold

A- Electrospinning with rotating metal mandrel
B- Thin mat of random PLLA fibers deposited on the surface of the metal mandrel
C&D- The resultant scaffold
E- SEM image of the scaffold's surface

Experimental condition:

Cathode metal mandrel (Diameter x Length)	Polymer	Solvent	Concentration	Flow rate (ml/h)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Cathode Rotation speed (RPM)	Spinning Time (min)	Fiber diameter (μm)
4mm X 250mm	Poly-L-Lactide Acid	Chloroform	15 % (W/W)	0.8	22	0.4	20	2	150	≈ 2

Chapter 5 (*Microtubes*)

Microtubes (Hollow Fibers)

Tiny Microscopic tubes have a variety of industrial and medical applications. They have the potential capability to be used in microfluidic devices and neuroscience research.

Axonal Guidance, Cell culturing, Membrane for hemodialysis machine, Bio sensors & devices, Heat exchangers, Liquid and particle separation, Optical sensors and device, Bragg sensors and photonic Crystals are just some examples of potential and already realized applications.

A prototype Microtube production system was built (**Fig.42 A, B**). The system creates and stabilizes a bubble of a polymer melt with air inside, then the bubble is spun and collected by the take up wheel. The presence of the air inside the bubble leads to the formation of a microtube during the spinning process. (**Fig.43 A, B**).

When bubble changes to droplet (no air inside), then instead of tube, micro fiber will be produced.

Tubes & Fibers are capable of carrying mass and energy, mass in the form of liquid or liquid-nanoparticles and energy in the form of electricity, heat or electromagnetic radiation and light (**Fig.45**). In case of some modifications, glass and a wide range of polymers (melt or solution) can be spun. Tubes and fibers can have composite structures, surface porosity with multiple holes and different cross sectional shapes. (**Fig.44**)

Fig.42 A. Microtube production system

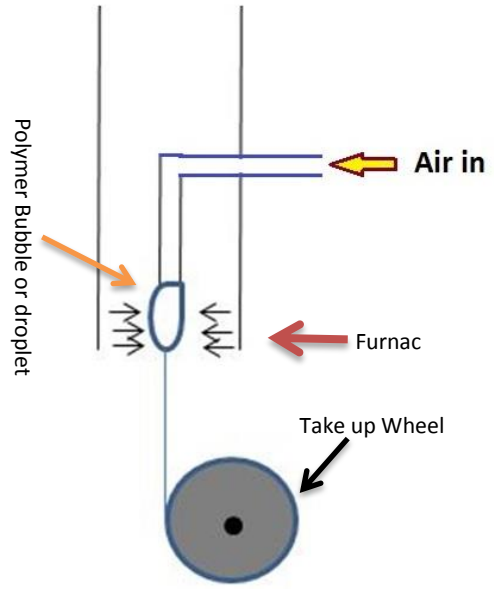


Fig.42 B. Simple diagram of the Microtube production system (technical details are not shown)

Fig.43 A. Polystyrene microtubes collected on take up wheel

Fig.43 B. Polystyrene microtubes

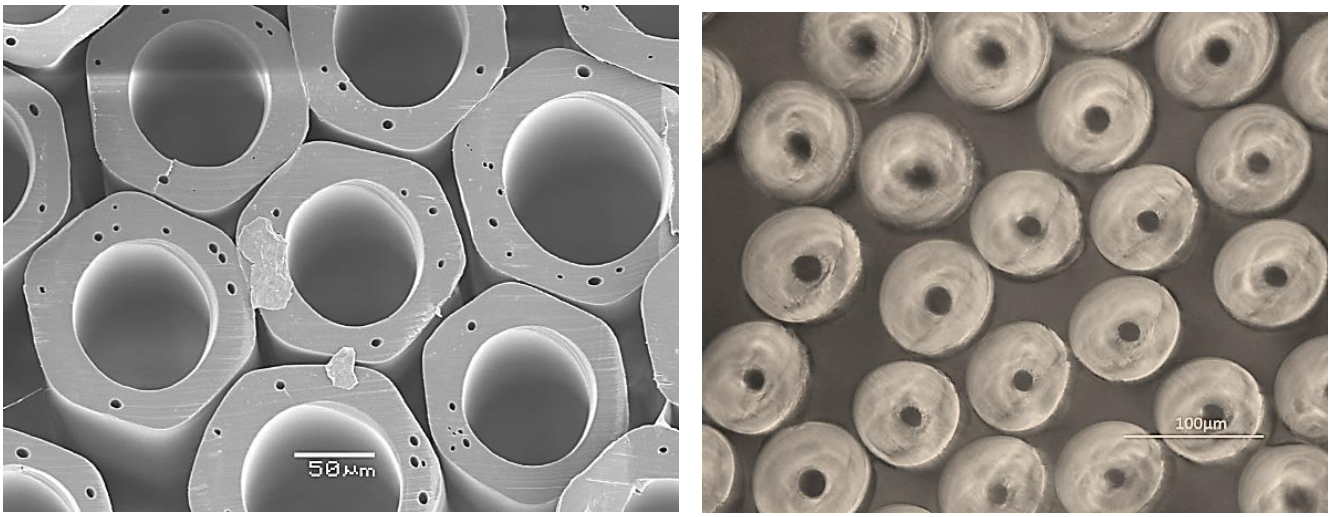


Fig.44. Polystyrene microtubes

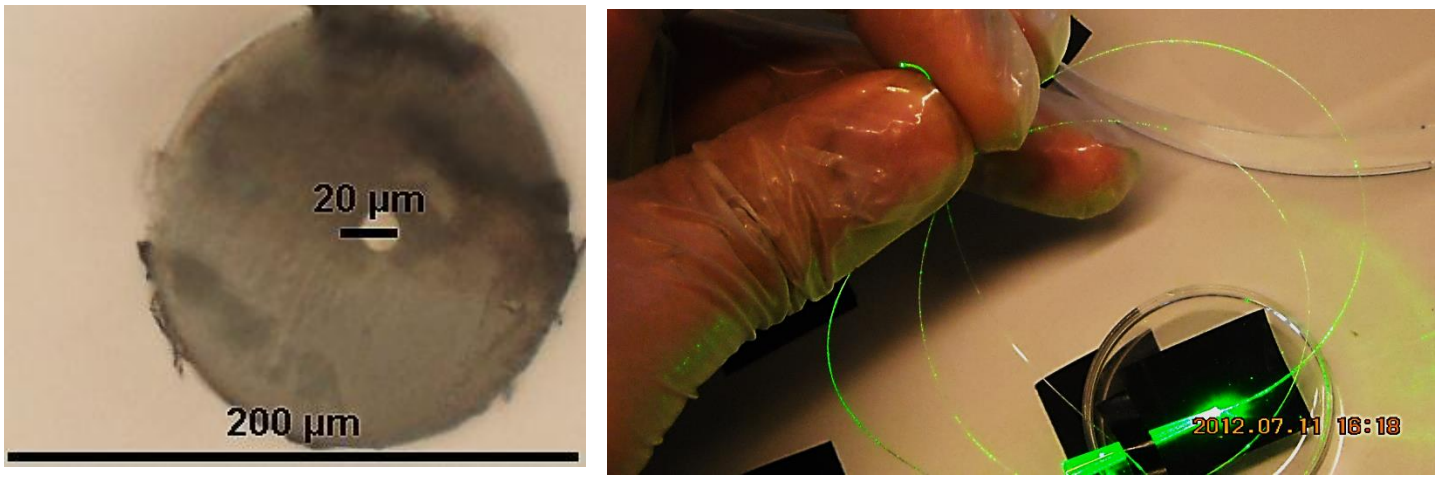


Fig.45. Right image shows a polystyrene microtube that acts as a light guide. Left image shows the cross section of the same tube

Microtube assemblies:

Thousands of tubes together make a hollow fiber assembly (**Fig.46**). Hollow fiber assemblies are used as membrane in hemodialysis machine and in bioreactors for cell culturing and the production of proteins and biological agents like antibodies. Several types of these assemblies were produced during this master work.

Fig.46. Polystyrene microtube assembly, The tubes in the two ends of the assembly are connected together with Polydimethylsiloxane (PDMS). Inset (SEM image) shows a cross sectional view of one end of the assembly.

Hollow fiber cell culturing (Bio Reactor):

In comparison to the regular culture dishes like T flasks, due to the high aspect ratio of the microtubes, an assembly of the hollow fiber (tubes) can provide a higher cell culturing surface area in a small volume of space. This large surface area allows culture of the cells at higher density and as a consequence the harvest (cells, proteins, antibodies …) will be in a very high concentration. The cells are cultured on the outside surface of the tubes while the cell culture medium passes through the tubes and nourish the cells through the tubes walls. Since the waste produced by the cells and the glucose are small molecules, they can permeate through the tubes porosity into the tubes and leave the reactor. However larger molecules like antibodies, viruses, proteins cannot permeate into the tubes and remain in the shell portion of the reactor. As a result the concentration of these biological entities or agents increases in the shell portion and can be collected by harvesting valves **Ref. [16].** See **Fig.47-48**

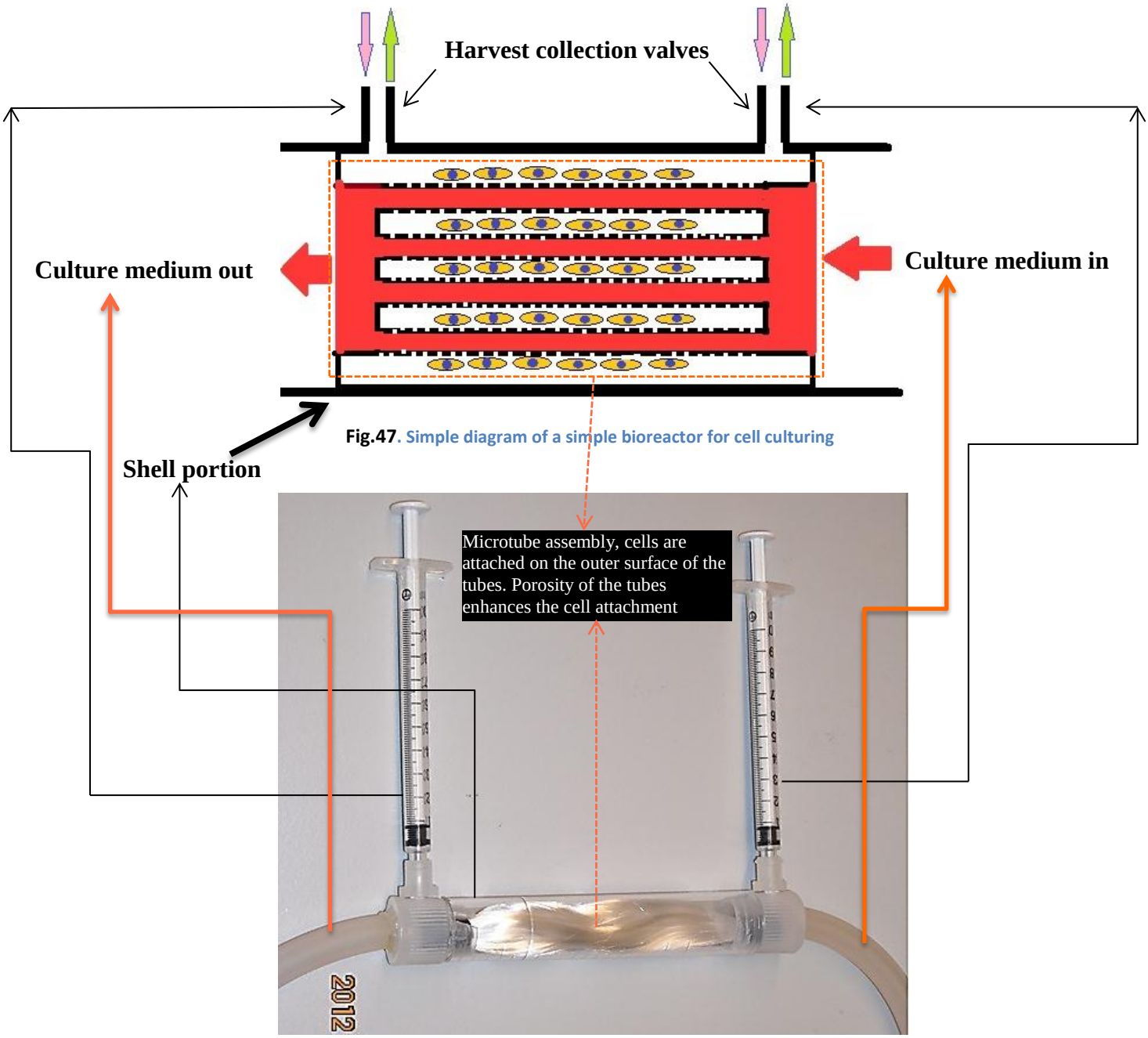


Fig.47. Simple diagram of a simple bioreactor for cell culturing

Fig.48. Sample bioreactor with a polystyrene microtube assembly was built during this master work

It is possible to give the tubes magnetic or optical properties or make them conductive or have a control over their porosity. These variations may make microtube assemblies interesting devices on nerve regeneration and stem cell research experiments.

Chapter 6 (*Axonal Guidance, Axonal stretching*)

Capability of electrospun fibers and microtubes on guiding the axons is shown in this chapter. A conventional Axonal stretching method is also briefly discussed and an imaginary experiment with a vibrating sample net consisting of aligned magnetic EVA ultra-fine fibers was conducted. The experiment shows that vibrating can have stretching effects on axons.

Axonal Guidance (Microtubes)

An assembly of the tubes was prepared and the free spaces between the tubes were filled with Polydimethylsiloxane (PDMS). The assembly was then cut into 2mm slices (**Fig.49-50**). Then dorsal root ganglions (DRGs) from an adult mouse mounted in Matrigel were put on top of the tubes in the center of the slices (**Fig.51**). Serum-free culture medium was added and the samples were incubated for 3 days. Then the samples were fixed and stained (Appendix 1). Samples were observed in a Fluorescence Microscope. Results show axons go inside the tubes occasionally (**Fig.52**). This indicates that microtubes can guide the axons. This may bring new opportunity on designing new implants for nerve regeneration and repair and new hope for nerve damaged patients. More advanced structures are possible.

2mm- thick slice

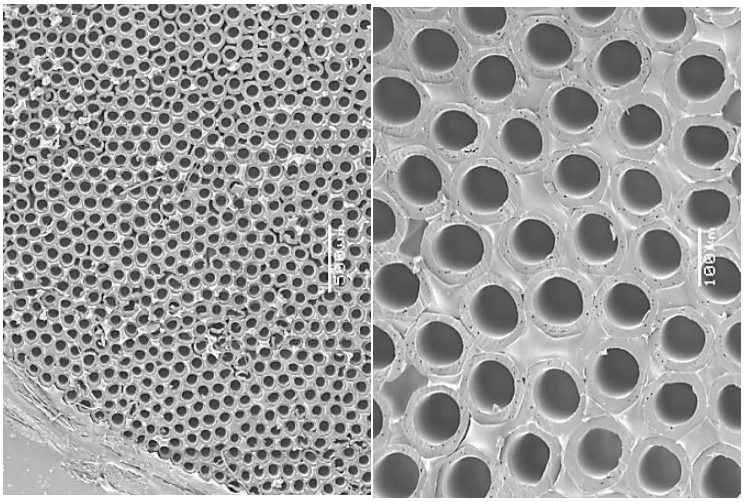


Fig.49. Assembly of microtubes

Fig.50. SEM images- Slice surface, More advanced structures are possible

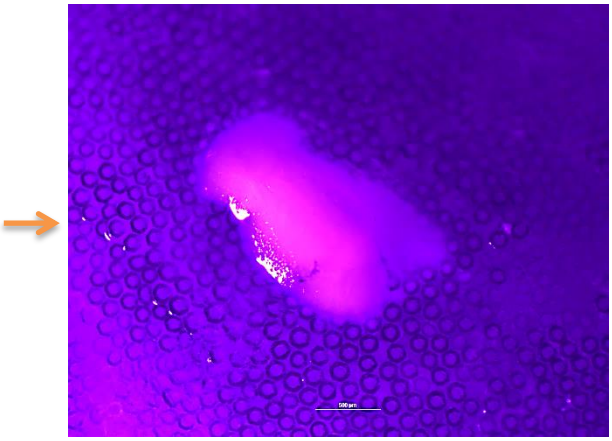


Fig.51. DRG on top of the Microtubes

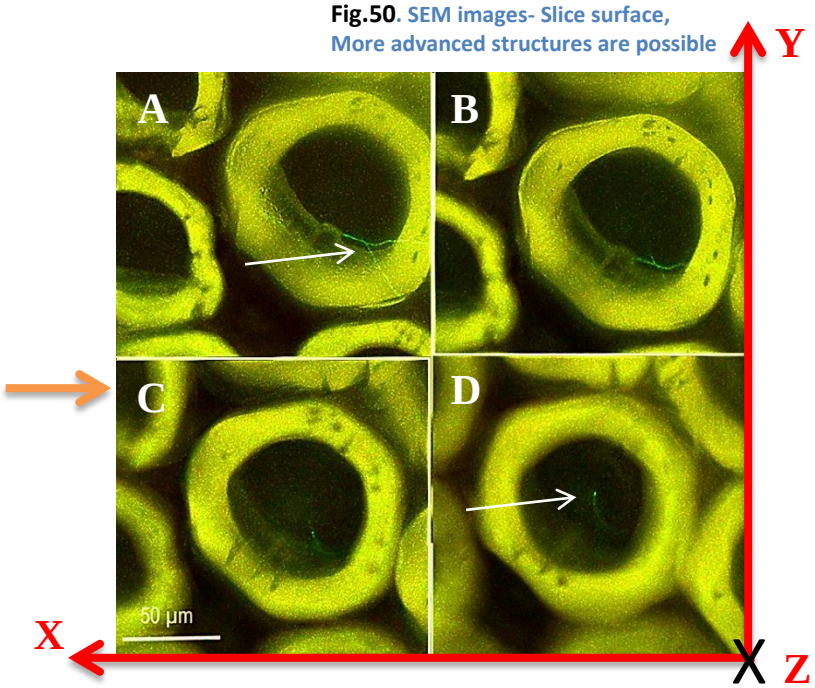


Fig.52. Microtube guides the axons.

In order to trace the axons inside the tube, focus of the microscope was changed gradually. 4 images were taken. Total Image displacement in Z direction (between image position in A and D) is around 100 µm. However it is expected that the axons traveled down in the tubes more than what I just measured by changing the microscope focus.

A) Two axons enter into the tube
B) The same axons cross each other inside the tube
C) One of the axons attaches to the wall of the tube and disappears.
D) The other axon still is visible in the center of the tube.

Axonal Guidance *(Micro Fibers - Gap method)*

Using gap method **aligned PLLA fibers** were spun, collected and fixed on cover slips. (**Fig.53**)
Then dorsal root ganglions (DRGs) from an adult mouse mounted in Matrigel were put on the aligned fibers. The entire preparation then was put in tissue culture dish containing serum-free culture medium supplemented with nerve growth factor (NGF) (**Fig.54-55**). After an incubation period of 3 days, the samples were fixed, stained and mounted on microscope glass slides (Appendix 1). Samples then were observed in a Fluorescence Microscope. Results show aligned fibers guide the axons nicely (**Fig.56**).

Fig.53: Parallel PLLA fibers ready for culturing

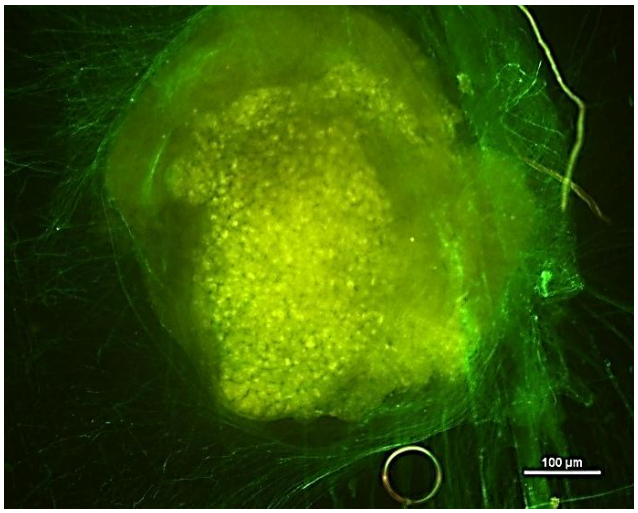


Fig.54: Dorsal root ganglion (DRG)

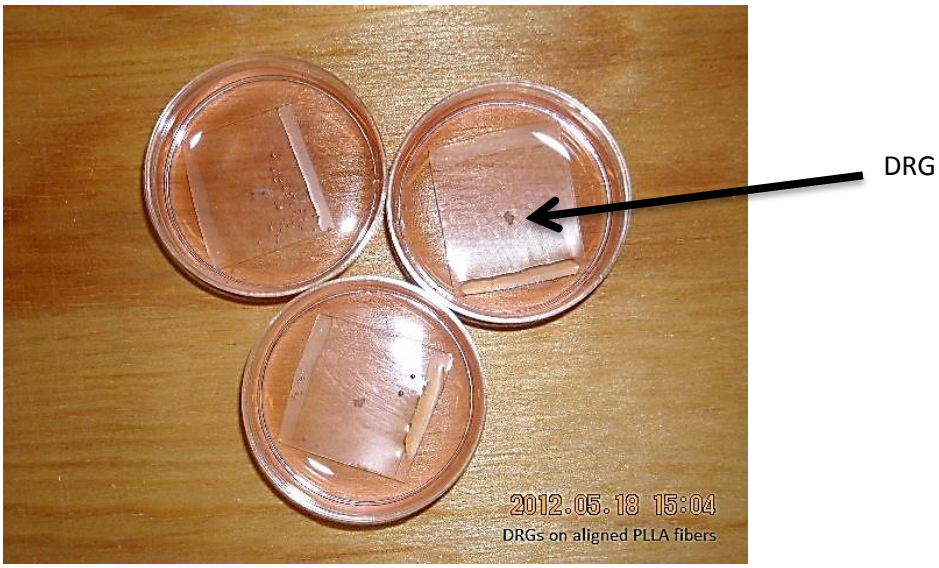


Fig.55. DRGs cultured on the aligned fibers in serum-free culture medium.

Experimental Conditions:

Polymer	Solvent	Concentration	Flow rate ml/h	HV (kv)	Needle Diameter (mm)	Distance between Needle-Collector (cm)	Spinning Time (min)	Fiber diameter (µm)
Poly-L-Lactide Acid	Chloroform	15 % (W/ W)	0.4	22	0.4	20	25	≈ 2

Fibers and axons **Axons**

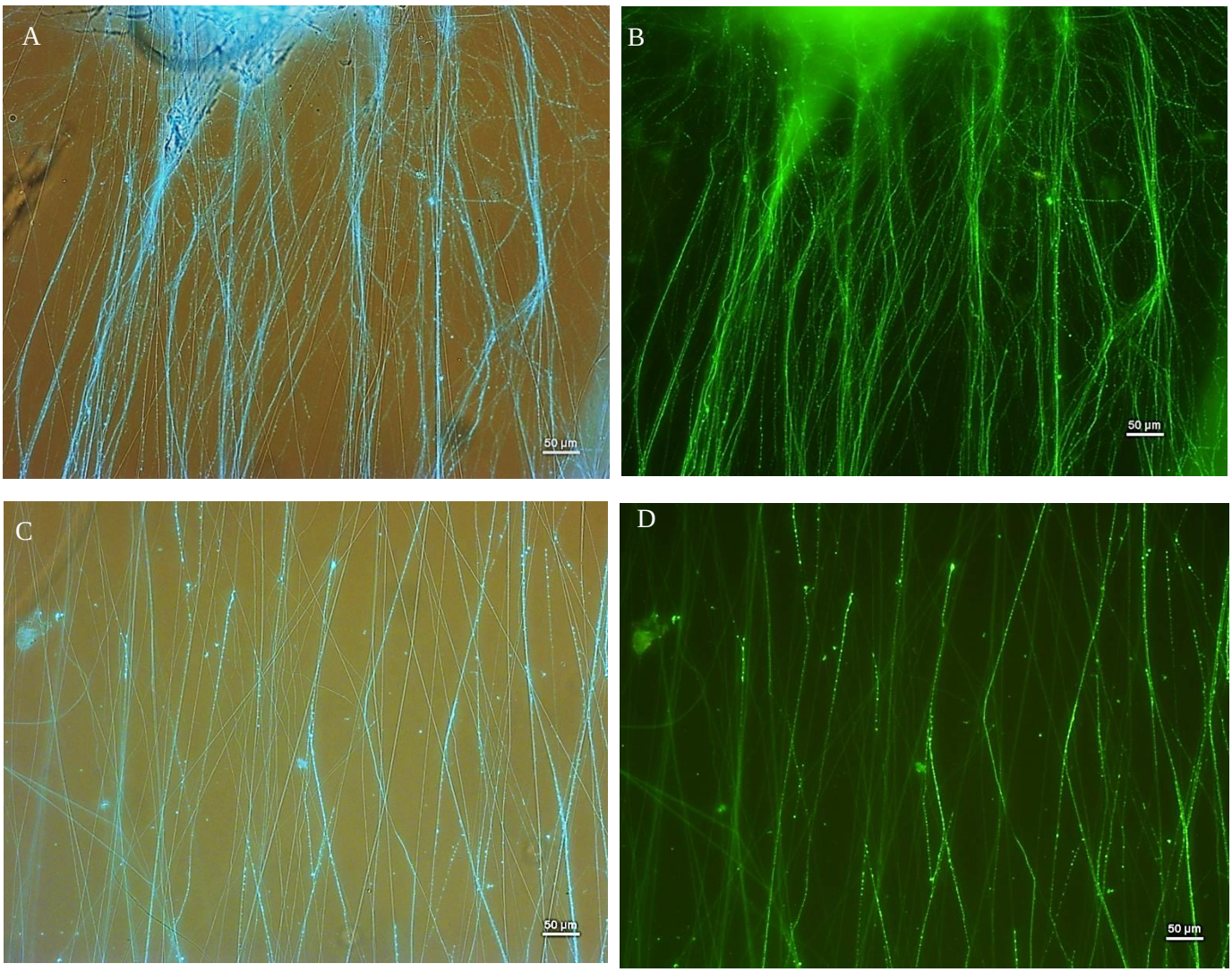


Fig.56. Images show axonal growth along the electrospun PLLA fibers. (ß3-tubulin staining).
-Right images were taken under UV light. Green fluorescence lines represent the Axons (Nerve fibers)
-Left images are the same images were taken under phase contrast + UV light. PLLA fibers and the Axons clearly can be identified.
Comparison between the images in the right and the images in the left clearly reveals that axons prefer to follow the fibers.
(Scale bar in all images is 50µm)

Axonal Guidance *(Micro-Nano Fibers - Rotating disk)*

PLLA aligned fibers were spun on conductive coverslips mounted on the surface of a rotating disk (Fig57, Type1, A) and on a plastic film band wrapped around the edge of the same disk (Fig57, Type2 D). DRGs from an adult mouse were cultured on the fibers. After 3 days of incubation, the samples were fixed, stained and mounted on microscope glass slides. Samples were observed in a Fluorescence Microscope. Results show PLLA aligned fibers guide the axons very nicely (**Fig. 57 C, F**). In comparison, the fibers from the edge of the rotating disk are more aligned than the fibers from the surface of the disk and higher the fiber alignment is, the higher axonal alignment will be.

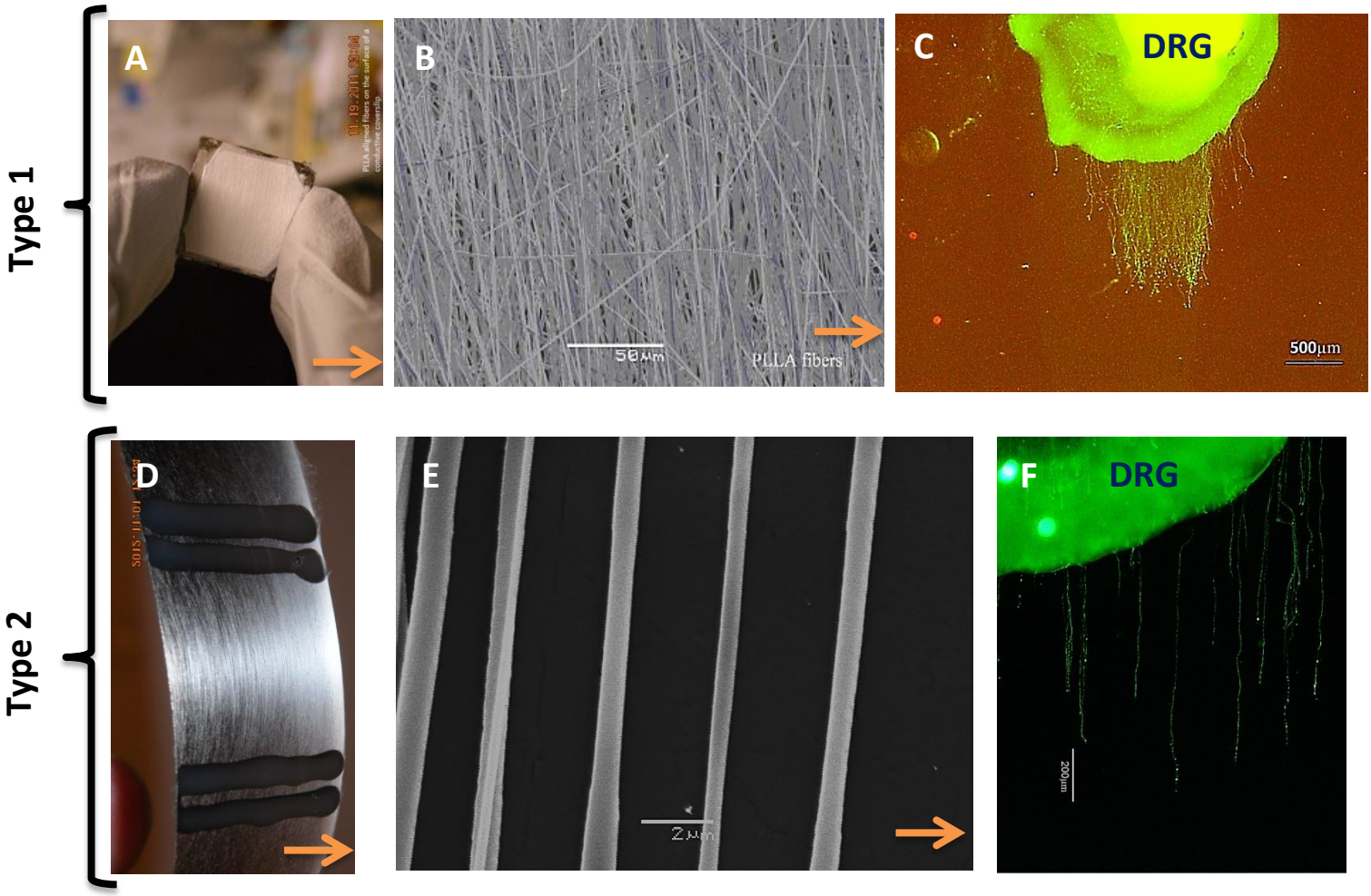


Fig.57. PLLA aligned fibers guide the axons

Type 1 :

A) Densely packed PLLA aligned fibers on conductive coverslip (Gold- platinum coated coverslip)

B) SEM image of PLLA aligned fibers

C) Axonal outgrowth, guided by the aligned fibers

Type 2 :

D) Sparse PLLA aligned fibers on plastic film (PLLA)

E) SEM image of sparse PLLA aligned fibers

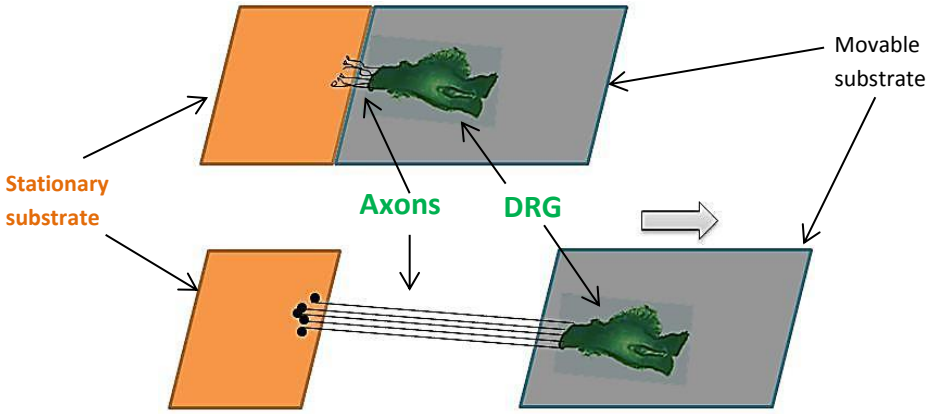
F) Axonal outgrowth, guided by the sparse PLLA aligned fibers

Experimental Conditions:

Type	Polymer	Solvent	Concentration	Flow rate (ml/h)	HV (kv)	Needle Diameter (mm)	Needle - Cathode Distance (cm)	Cathode Rotation speed (RPM)	Spinning Time (min)	Fiber diameter (µm)
1	Poly-L-Lactide Acid	Chloroform	15% (W/W)	0.8	22	0.4	20	5500	40	≈ 3
2	Poly-L-Lactide Acid	Chloroform	15% (W/W)	0.8	22	0.4	20	5500	20	≈ 0.5

Axonal stretching

Studies show that slow stretching of axons can increase their growth rate. In one study **(Ref.20),** in a specially designed bioreactor, two substrates were used, one stationary and one moveable. Dorsal Root Ganglia (DRGs) from an E16 rat pup was placed on the moveable substrate, near to the stationary substrate **(Fig. 58).** Bioreactor was filled with culture media and incubated for 5 days. During this time axonal processes were extend onto the stationary substrate and the growth cones were attached to the stationary substrate **(Fig. 58 top).** In the second part of the experiment, axons were stretched. Specially designed mechanism in the bioreactor could move the movable substrate with a very low speed. This caused stretching on the axons **(Fig. 58 bottom).** Over first 24 hours one mm stretch was achieved. However in 6th day, a stretch rate of 5mm/day was possible **Ref. [20].**

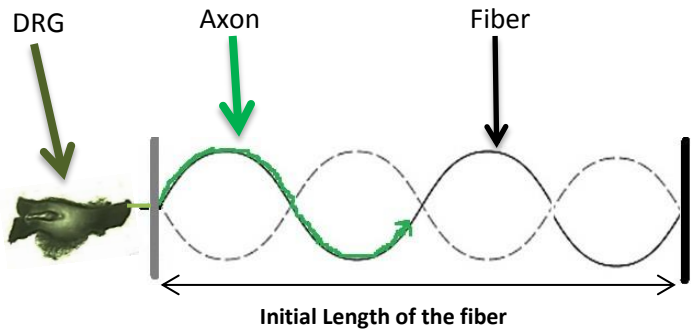


. **Fig.58.** Mechanical stretching of Axons.
Top: Axonal processes extend onto the stationary substrate.
Bottom: Movable substrate moves and the axons experience mechanical stretch.

This is a remarkable growth rate. However, in the experiment only the growth cones are adhered to the substrate and the axons between the two substrates are just floating and are vulnerable to damage during handling. In addition, the stretching itself is due to applying direct mechanical force and this in the end, leads to a large change in the system mechanics. I think, applying such a big change in mechanics will be challenging in in vivo applications.

Vibrating nerve regenerative implant

I think there may be a gentle approach to stretching axons. I propose that this gentle approach is the vibration of the axons. Vibration can cause stretching effects on the nerves and axons. It may be possible to vibrate the axons through the resonance effects. The vibration can take place in different ways, by applying sound waves for instance, like ultrasonic vibration or piezoelectric vibration or by applying a dynamic electromagnetic field when the axons are on a special micro-nano fibers or wires. A simple example of such a system is shown in **Fig.59.** In this imaginary experiment, the outgrowing axon from a DRG is guided by a fiber. The fiber is undergoing a vibration. Vibrational frequency and its wave function can be varied (a sinus function, in this example). Vibration travels along the fiber as a sine wave. The reflected wave interacts with the forwarding wave and the outcome is the standing waves along the fiber.



. Fig.59 . Fiber is vibrated while guiding the axon.
Vibration of the fiber is transferred to the axon directly.

Smart implants vibrate through a resonance effects or dynamic electromagnetic fields. As an example, a sample implant with aligned "EVA-Iron oxide nanoparticle" composite fibers suspended in the middle of a petri dish was produced during this master work **(Fig.60A).** Then the sample was immersed in distilled water **(Fig.60B)**. The implant can vibrate in a dynamic magnetic field. A simple calculation of the maximum fiber length extension (ΔL) in every vibrational sequence is presented in **Fig.61-C**. Assumption in the calculation is that; the curve L_2 is a part of a perfect circle.

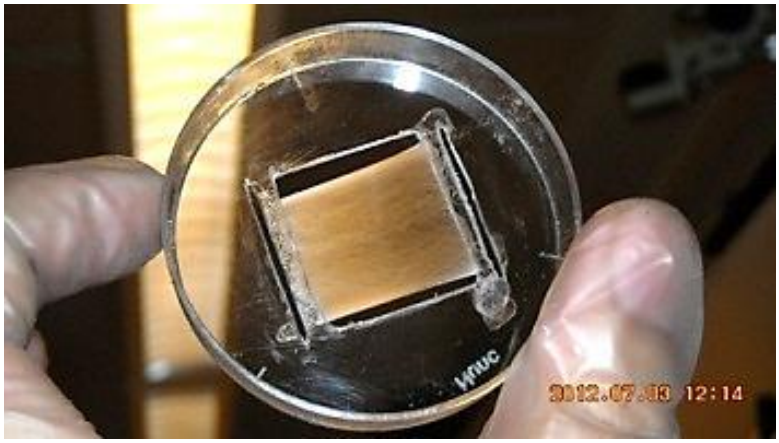


Fig.60 A. aligned "EVA-Iron oxide nanoparticle" composite fibers produced through the gap method and suspended in the middle of a petri dish

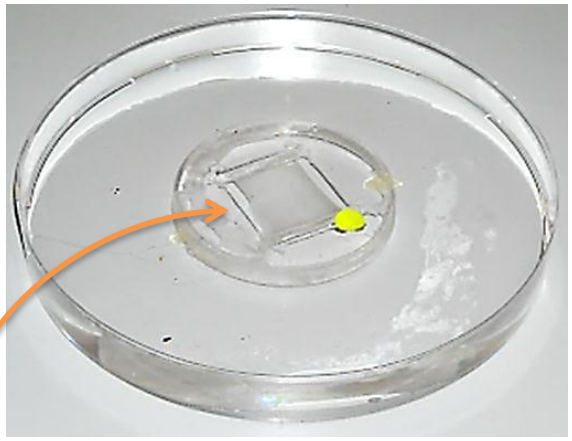
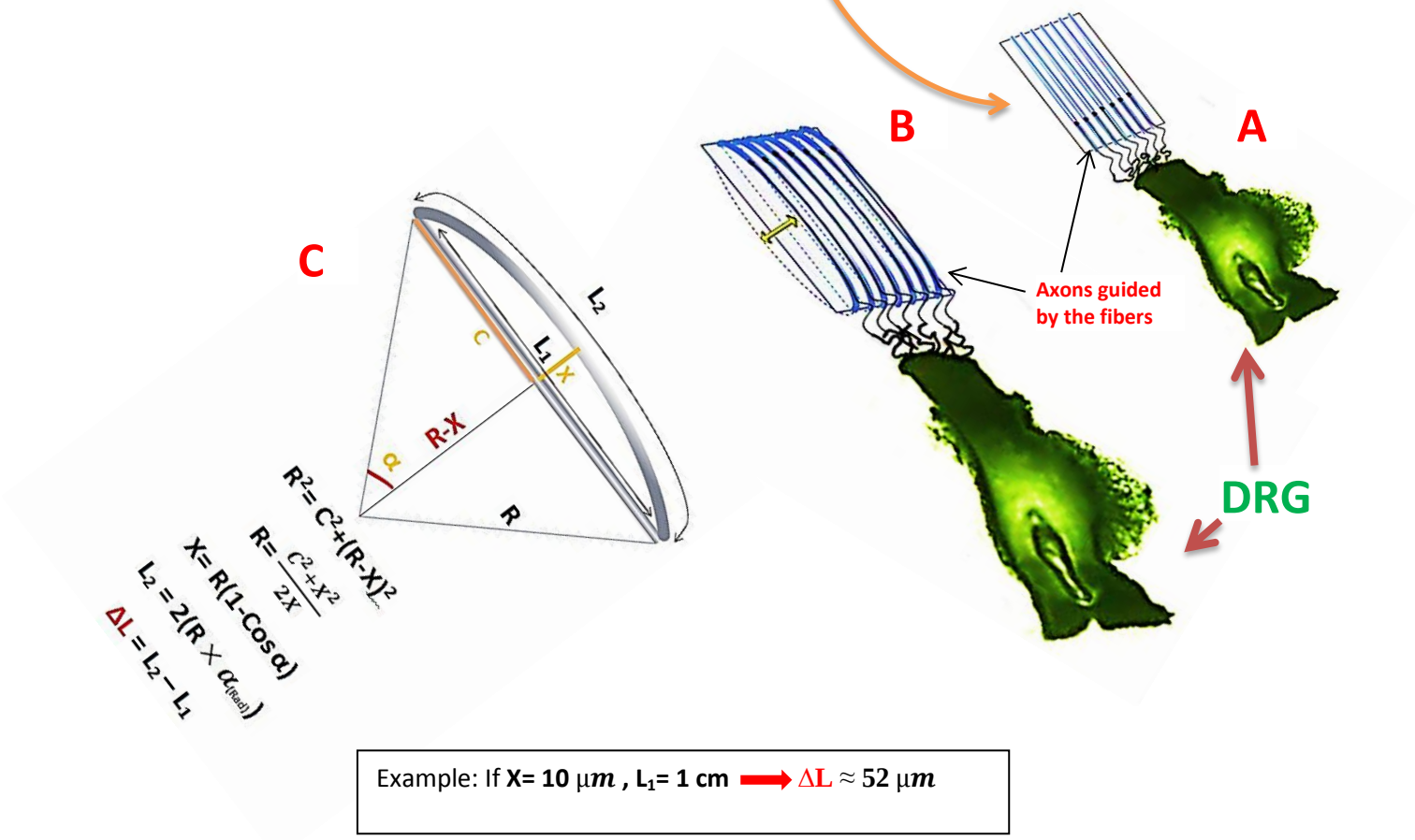


Fig.60B. The sample implant immersed in distilled water. This may mimic in vivo like environment.



Example: If **X= 10 μm , L_1= 1 cm** ⟶ $\Delta L \approx 52\ \mu m$

Fig.61. An imaginary experiment with a sample net consisting of aligned magnetic EVA ultra-fine fibers. DRG is cultured on one side of the net and the whole system is placed in a simple dynamic magnetic field (On and Off field)
A) The net guides the axons. Magnetic field is off
B) Dynamic magnetic field is applied. The net is vibrating.
C) Shows maximum fiber length extension ΔL in each vibrational sequence. **X** is proportional to the maximum intensity of the magnetic field.

Chapter 7 (*Micro wire Coating - Brain Machine Interface Electrodes*)

Micro wire Coating:

Nanofibers and beads can also be coated on the surface of microfibers or tubes. If the target is conductive (*e.g.* metallic micro wires and tubes), then the target itself can be considered as a cathode. In case of none conductive target (*e.g.* polymeric microfibers and tubes), a specially designed cathode is needed. In this case edge of an aluminum plate with thickness around 2mm is used. (**Fig. 62**) The edge of the plate is placed in parallel and just behind the nonconductive target (in here a polyimide insulated wire).

In electrospinning process, nanofibers or beads hit the insulated wire and accumulate on its surface just before they can reach the cathode. In other words the wire acts as a trap and stops the nanofibers and beads before they can reach the cathode. (**Fig. 62** B)
A variety of polymer nanofibers and beads including Polystyrene (PS), Poly-L-Lactide Acid (PLLA), Polyimide (PI) and Poly ethylene-co-vinyl acetate (EVA), with different morphology were spun on the surface of a polyimide insulated platinum Iridium micro wire. (See **fig. 63-66**)

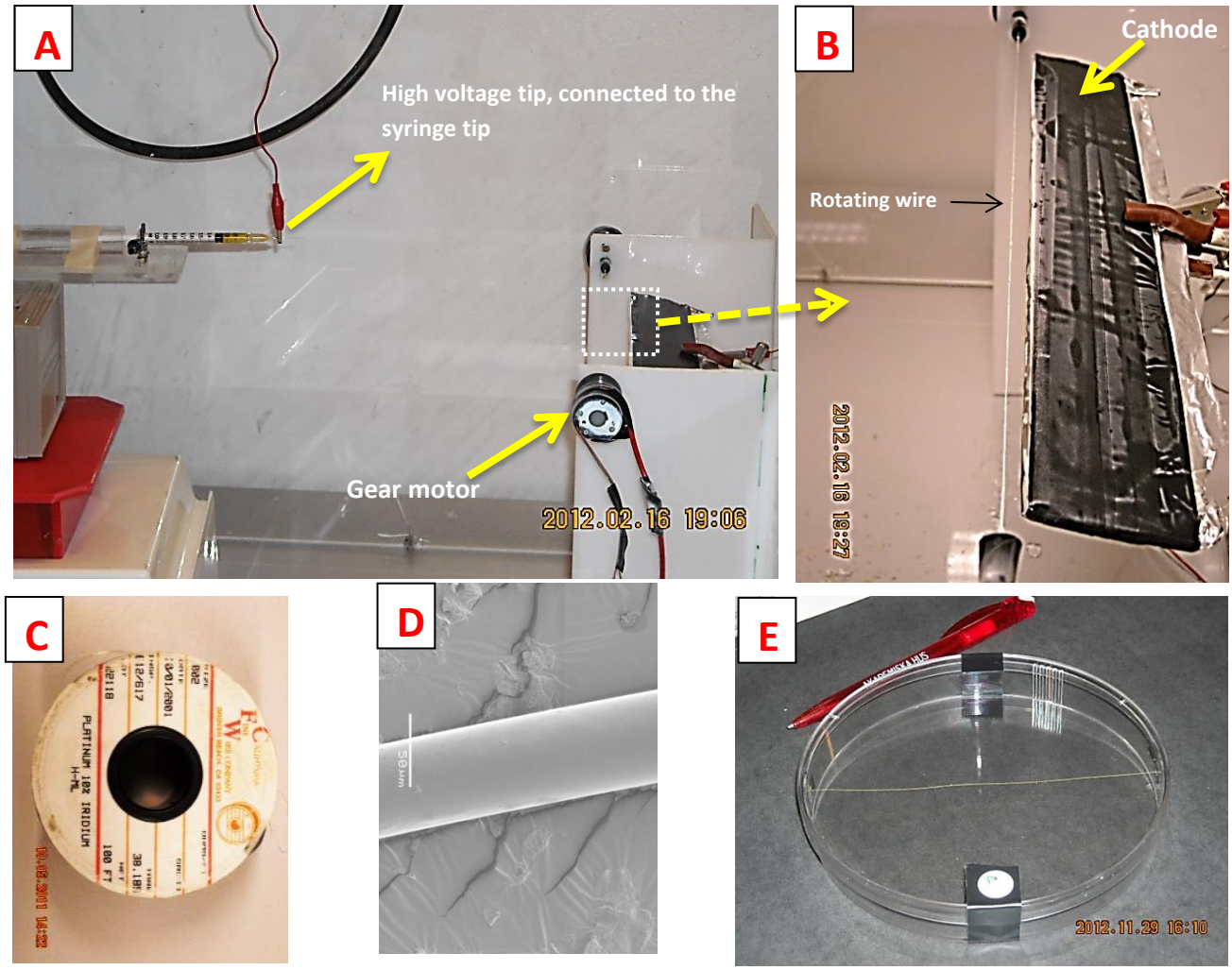


Fig.62. Micro wire coating,
A) the wire is slowly rotating in front of the cathode collecting nanofibers and beads on its surface. Rotation of the cathode results in a uniform coating.
B) Cathode is located in around 5 mm from the wire and just behind the wire.
C) Polyimide insulated platinum Iridium micro wire
D) SEM image of an uncoated wire
E) Coated wire suspended in a petri dish

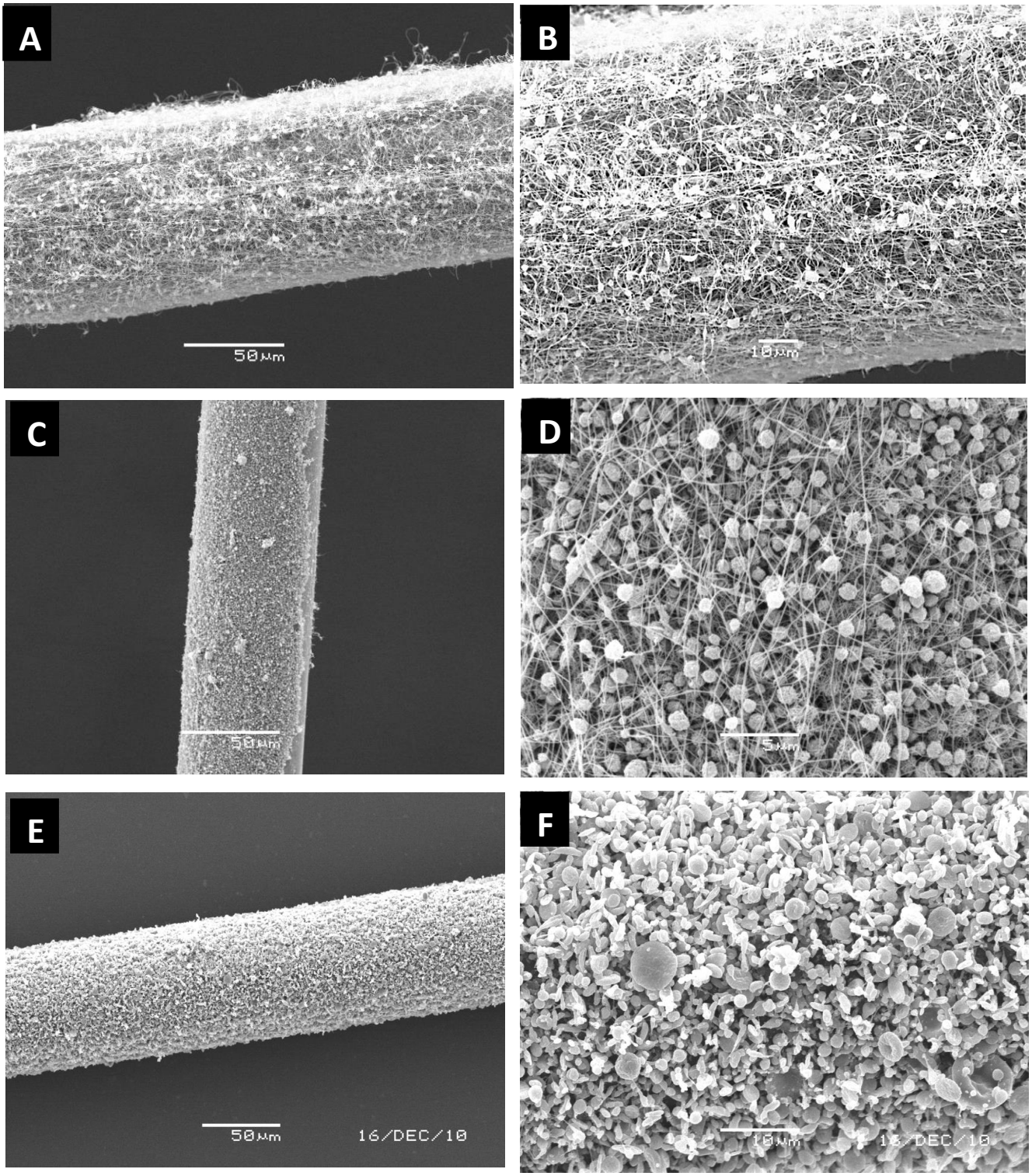
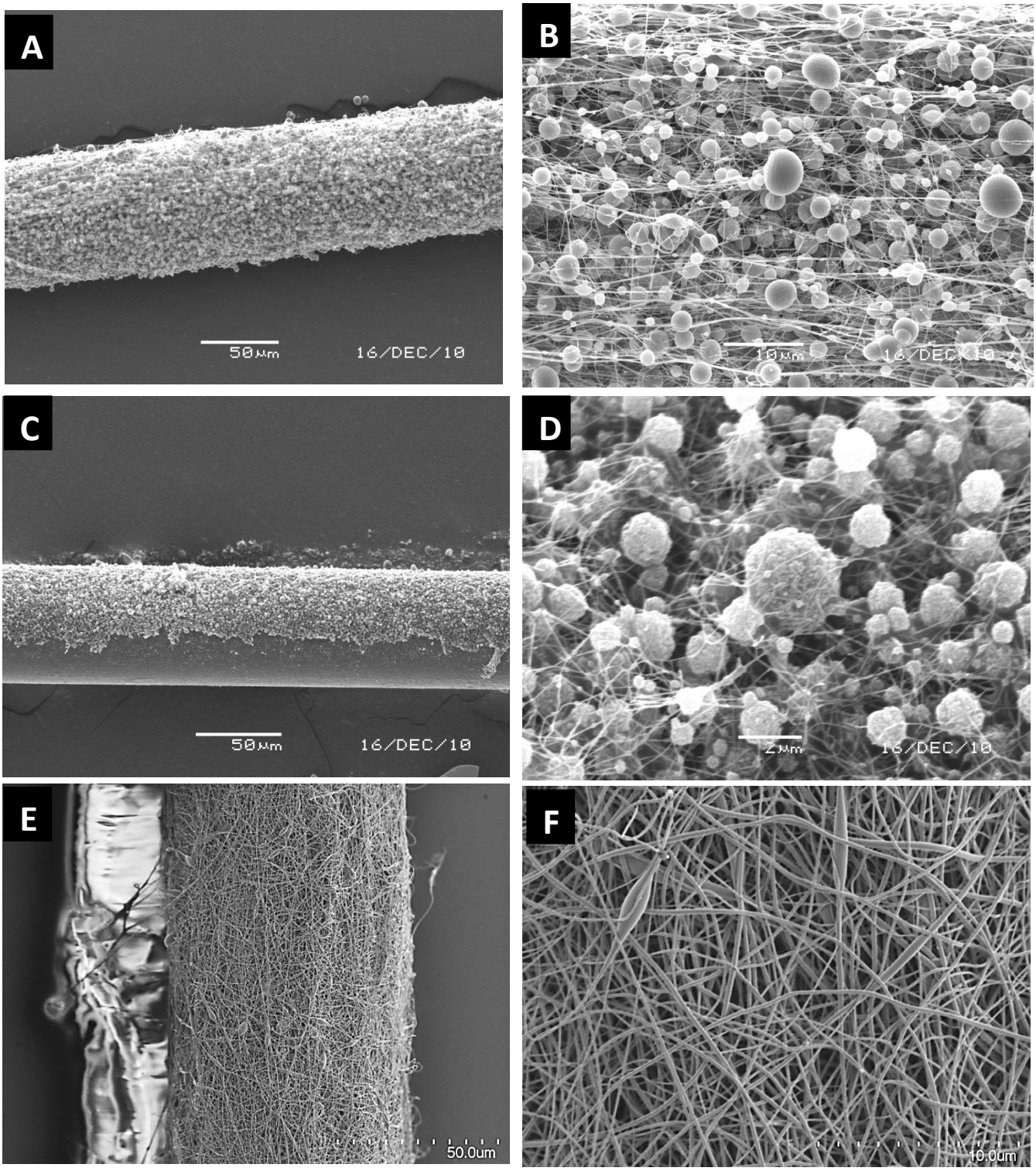


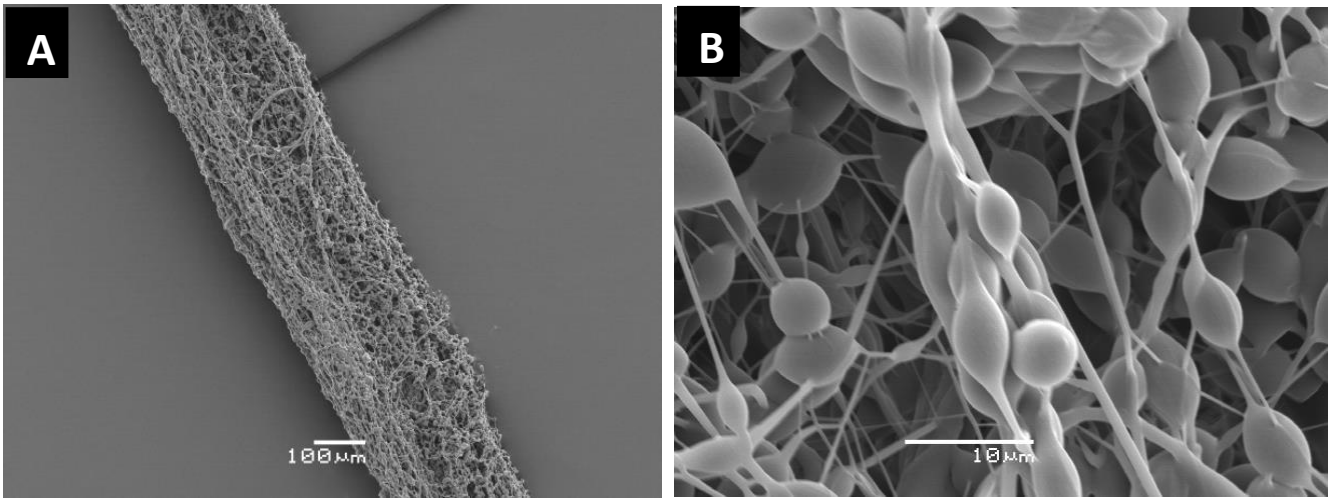
Fig. 63

A, B) SEM images of a platinum iridium micro wire coated with polystyrene nanofibers.
C, D) SEM images of a platinum iridium micro wire partially coated with polystyrene nanofibers and beads.
E, F) SEM images of a platinum iridium micro wire coated with polystyrene micro-nano beads.



- **Fig. 64**

A, B) SEM images of a platinum iridium micro wire coated with Poly-L-Lactide Acid nanofibers & micro beads.
C, D) SEM images of a platinum iridium micro wire partially coated with Poly-L-Lactide Acid nanofibers & micro-nano beads.
E, F) SEM images of a platinum iridium micro wire coated with polyimide nanofibers. See the corresponding 3D image in Appendix 2. (Fig.74)



- **Fig. 66**

A,B) SEM images of a platinum iridium micro wire coated with poly (ethylene-co-vinyl acetate) nanofibers and beads.

Experimental Conditions (Fig. 63-66)

Polymer	Concentration	Flow rate ml/h	solvent	HV (kv)	Needle Diameter (mm)	Needle-Collector Distance (cm)	Wire Rotation Speed (RPM)	Spinning Time (min)	Fiber diameter (nm)
Polystyrene (Fig 63 A,B)	≈3.5% W/W	0.9	DMF	35	0.7	35	3	10	≈150
Polystyrene (Fig 63 C,D)	≈2.6% W/W	0.9	DMF	30	0.7	32	0	5	≈100
Polystyrene (Fig 63 E,F)	≈1% W/W	0.9	DMF	30	0.7	32	3	10	Only beads!
Poly-L-Lactide Acid (Fig 64 A,B)	≈3.22% W/W	0.9	Chloroform	30	0.7	32	3	10	≈150
Poly-L-Lactide Acid (Fig 64 C,D)	≈1.23% W/W	0.9	Chloroform	30	0.7	32	0	2	≈50
Polyimide (Fig 64 E,F)	25% W/W	04	DMF	30	0.4	20	3	10	≈100
Poly (ethylene-co-vinyl acetate) (Fig 66 A,B)	7% W/W	0.8	Chloroform	35	0.4	10	3	5	≈150

Brain Machine Interface Electrodes

Background:

Brain stimulation has gained more attention ever since 19th century. Researchers noticed that muscles and nerves can be excited electrically. Stimulation of the brain along with recording the neural activities may bring new hope for paralyzed people or cure Parkinson disease and reduce depression. It may be an effective remedy for pain relief and may enable the patient to control prostheses or a paralyzed limb with sensory feedback. (**Fig. 67**)

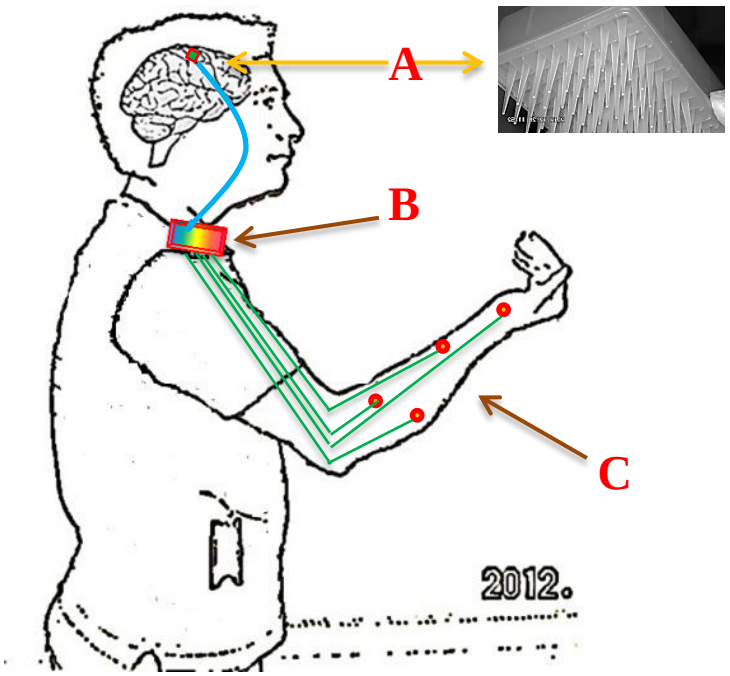


Fig. 67: An implanted array of electrodes (A) is connected to an external computer (B) and a paralyzed limb (C) in series. Neural signals are captured by the electrodes and sent to an external processing unit outside the brain. The Action potential signals from the neurons are being analyzed and changed into electrical pulses that can stimulate the paralyzed limb or control the prostheses or an external device.

Parkinson disease is a consequence of Substantia Nigra cells degeneration. The neurons in this area produce dopamine. These neurons are known as dopaminergic neurons. Dopamine is a neurotransmitter that plays a very important role in regulating variety of the physiological functions. Signaling between dopaminergic neurons and the different parts of the brain takes place in the presence of dopamine. The signals from these cells that are sent to the different parts of the brain help the brain to control the movement of the body smoothly.
When degeneration occurs, signaling is not normal and this abnormality in signaling will make different regions of the brain overactive. This over activity will cause difficulties in body movement. Implanting special electrodes in the brain can help to control the "over activity" in different regions of the brain and bring them to their normal active state. This will help the patient to move in a more normal way. These electrodes are known as Deep Brain Stimulation (DBS) electrodes. (**Ref: 27 & 28**)

Brain Stimulation technique was improved when "Victor Horsley "(1857–1916), a neurosurgeon invented a three-dimensional coordinates system (Stereotactic surgery) for putting small objects inside the body. The technique enables the surgeon to perform several actions like biopsy, injection and stimulation. Another major development that gave the brain stimulation a boost was made by the Swiss neurophysiologist Walter Rudolf Hess (1881–1973) when he developed the chronic electrode implants. Scientists are trying to develop different electrodes with different materials, shapes and sizes and different insertion methods. The ultimate aim is to develop electrodes that elicit a minimal foreign body reaction. (**Ref: 21).** However this is still a challenging task to be met.

Brain Tissue

Neurons approximately make up 25% of the cells population in the brain tissue. The rest consists of the vascular tissue and Glial cells.
Glial cells are consisting of three cell types including:

1) ***Oligodendrocytes*** : that insulate axons and form myelin sheaths.

2) ***Astrocytes***: around 30-65% of the glial cells consist of astrocytes.
Astrocytes have star like shapes. They supply the neurons with growth cues during the development of the Central Nervous System (CNS). They act as a mechanical supporter for the neural networks. Buffering the neurotransmitters and releasing the ions in neuronal signaling are also known to be done by this type of glial cells. Normal astrocytes can transform into active mode due to an injury or wound. Active mode means enhancement in proliferation, migration, hypertrophy and up regulation of polymerized glial fibrillary acid protein (GFAP). It also means a change in cellular organelle distribution, deposition of glycogen and *an increase in matrix production*. The astrocytes are responsible for the formation of Glial scars (encapsulation) after injury to the CNS.

3) Microglia: These cells make up around 5-10% of the glial cells. After an injury they engulf and kill pathogenic organisms or release enzymes that can break down damaged matrix and cellular debris. They increase in number when they transfer to an active mode. In order to help break down the external objects, they release more lytic enzymes. Microglia cells release several factors that influence different processes as well as signaling pathways, so as a consequence, this makes it very complex to find out their exact role in brain response to implanted electrodes. **(Ref: 22)**

Failure of Neural Electrodes in Chronic Tissue Response

Astrocytes & Microglia cells are activated in response to the implanted electrodes.
While an implanted neural electrode can work well early after implantation, it often fails to perform well during long term experiments. It seems that the electrodes fail mainly due to *glial encapsulation*.
Astrocytes & ***Microglia*** cells are responsible for encapsulation of the electrodes.
When encapsulation happens, the electrodes simply are isolated and the scar tissue pushes the neurons away from the electrodes. This means that no more electric signals can be transmitted through the encapsulated electrodes and the electrodes fail. **(Ref: 22)**

Coating neural electrodes!

Coating the electrodes with a mixture of fibers and beads can mimic the extra cellular matrix.
This may help to hide the electrodes from body immune system (*Camouflage*!) and reduce the foreign body reaction.
Fiber coating may result in a better and flexible anchoring of the electrodes in the brain tissue and perhaps will result in less tissue inflammation. Fibers and the beads can also be loaded with anti-inflammatory drugs.
Fibers with a high aspect ratio (Surface/volume) will release their drug faster and in a shorter period of time, while the beads that are large, will release their drug during a long period of time. (Months- years)

In a group work and in a joint project between our LAB and Neuro-nano Research Center (NRC), micro electrodes were coated with polyimide nanofibers and implanted into the brain cortex of rats. The aim is to see how the coated surfaces affect the inflammatory response. The project is still ongoing, and the full report will be published soon.

The project steps:

- Surface toxicity test: Several glass coverslips were coated with polyimide nanofibers or covered with thin film of the same polymer. Some of the samples were baked at 220 C for 90 min. Baking results in full polymerization of the polyimide and the removal of residual NMP (N-Methyl-2-pyrrolidone) that is toxic. Macrophages were cultured on the surface of the baked Samples. No toxic effects were observed.
- Nanofibers (or thin film of polymer) were coated on Micro electrodes. The coated electrodes were then baked (**Fig.68**)
- Baked micro electrodes implanted into the brain cortex of rats. (**Fig.69**)
- Evaluation time of 6 and 12 weeks was applied.
- Brain tissues were sectioned (**Fig.71**)
- Evaluation techniques will be applied. (Immunostaining will be used. This has not been done yet)

Immunostaining:

ED1 (Rat macrophage-specific antibody),

In comparison with Microglia cells, macrophages have round and larger cell bodies with a diameter around 10-15 µm. Macrophages are ED1 immunopositive while Microglia cells are ED1-negative. (Ref: 24)

NeuN (Neuronal Nuclei),

Neurons are mainly NeuN positive and can be identified by NeuN immunoreactivity in cultured or sectioned tissue. (Ref: 25)

GFAP (Glial fibrillary acidic protein),

This protein is used as a cell marker and can be used to identify the astrocytes. (Ref: 26)

The hypothesis:

ED1 staining: It is expected that the concentration of macrophages around the control electrodes be higher than the fiber coated electrodes.

NeuN staining: Neurons can be identified around the electrodes. A distance between the neural population and an electrode can be a sign of encapsulation. The scare tissue pushes the neurons away from the electrodes.
It is expected that the distance of the neurons from the control electrode be longer than the coated electrode.
This means that less foreign body reaction around the fiber coated electrode is expected.

GFAP staining: Since Astrocytes are positive for GFAP, their activities (formation of the scar tissue) around the electrodes can be identified. More scare tissue is expected around the control electrode than the fiber coated one.

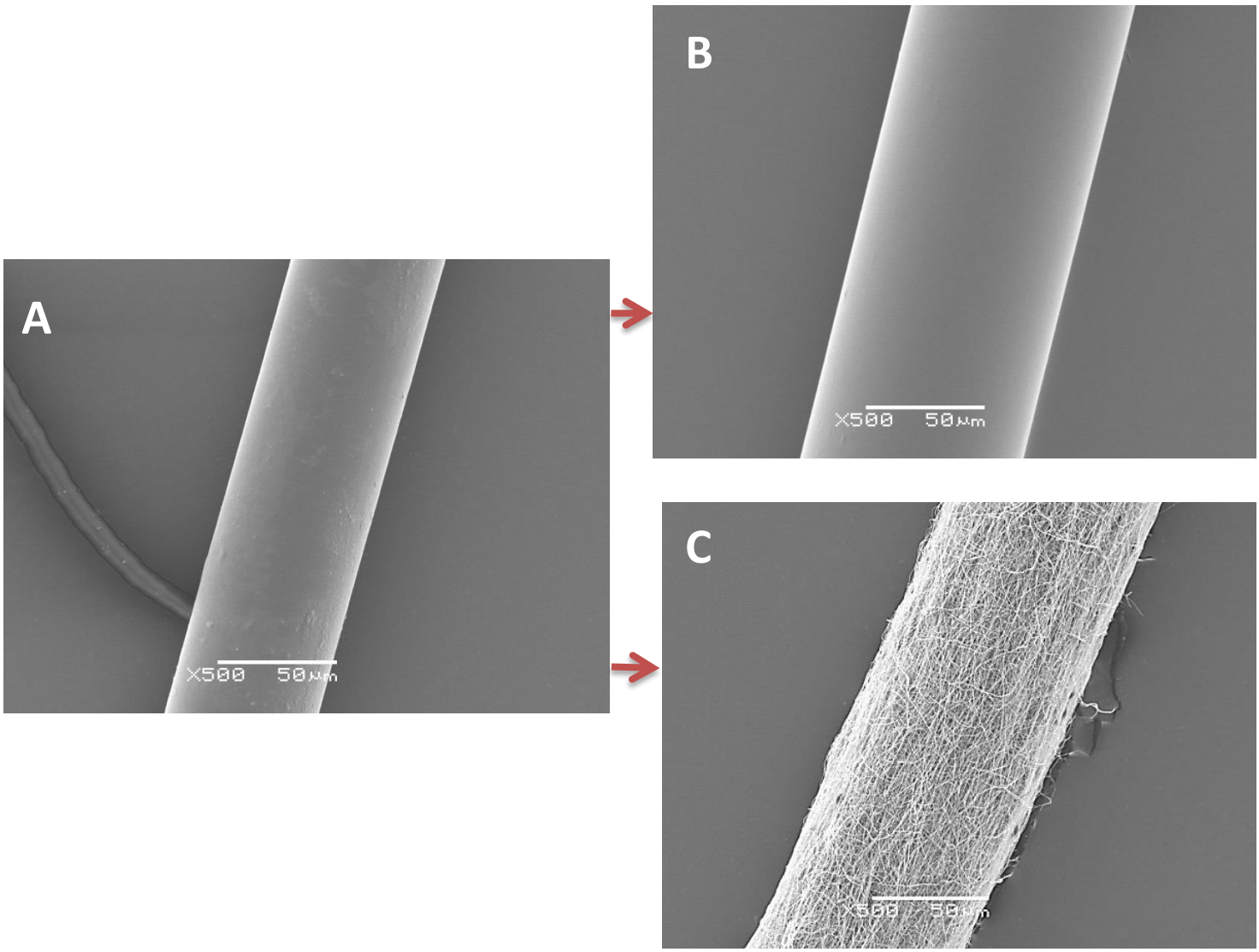


Fig.68. Uncoated & Coated Micro electrodes

A) Platinum Iridium micro electrode with Polyimide insulation
B) The same micro electrode coated with a thin layer of polyimide thin film.. This electrode is used as a control electrode.
C) The same micro electrode coated with polyimide nanofibers

Fig.70: Rats after implantation

Fig.69. Electrode insertion into the brain cortex of a rat, insertion took place using a micromanipulator.

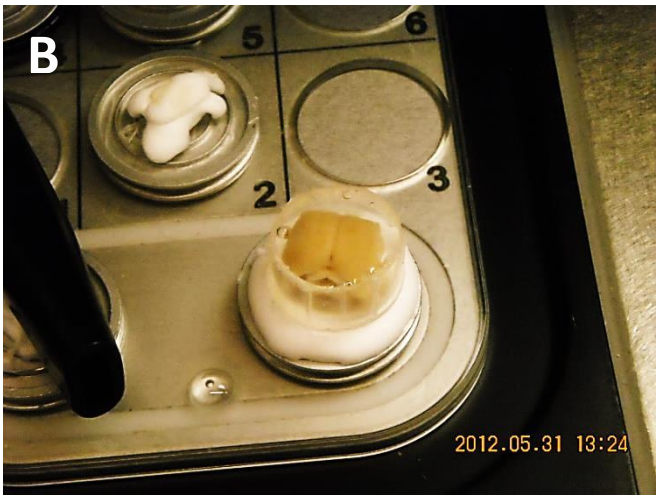


Fig.71. Cryostat sectioning

A) Fixed brain tissues from rats immersed in sucrose and ready for sectioning process. Sunken tissue is a sign of full impregnation with sucrose which took place in 5 days.
B) Tissue freezing, tissue was frozen and sectioned on a cryostat. Cryoprotectant (Sucrose in here) protects the tissue by preventing the formation of ice crystals during tissue freezing.
C) Frozen tissue
D) Cryostat Sectioning System
E) Sectioned tissue

Chapter 8 (*Conclusion*)

Conclusion

Networks of Nano-Micron size fibers and tubes that I work on in this project can provide a true 3D environment with the capability of mimicking Extra Cellular Matrix (ECM) for supporting cellular life and Cell-division cycle with a good cell-fiber adhesion property and the possibility of high density cell culturing. They are capable of cellular differentiation, guiding axons, migrating cells and other cell extensions, guiding light, carrying electricity and liquid, drug release, exchanging heat, become magnetic and vibrate in dynamic electromagnetic and sound fields. These networks have the potential to be used in a variety of biomedical applications especially in **Nerve regenerative implants** and **Neural electrodes**. Combing light with these networks can bring new concepts to these fields and may open a new window in neuroscience for diagnosis and treatment of neurological disorders and diseases.

Novel Implants

Some of the implants and applications on Nerve regeneration, Neural electrodes and Vision research that were developed at our center during this Master work, are unique and the novelty of some of them has been confirmed by the Lund University Innovation System (LUIS):

- A special neural optoelectrode
  Some elements of this electrode have already been made. Project needs financial support
- Implant for Restoring Sexual Potency after PROSTATECTOMY
  We have the proof of concept for this invention and several sample implants have been produced. Project needs financial support and has the potential to be commercialized

Products and applications with commercialization potential:

1) Nanofibers and Nanofiber Yarn for High-tech applications including textile industry (with diverse morphology, diameter and combinations, e.g. See page 20, Fig 25)
2) Wide range of polymer made Microtubes and Microfibers (with diverse morphology, diameter and combinations)
3) Nano Coating: Nanofibers, beads and thin polymer films can be coated on microwires/microfibers made of any kind of materials. (e.g. See page 45, Fig. 63 , page 46, Fig.64 & page 51 Fig.68 B, C)
4) Prototyping nano-micro fiber based scaffolds for tissue engineering (e.g. Blood vessel scaffold, page 34, Fig.41)
5) Production of continuous metal nanotubes and nanofibers (e.g. See page 26, Fig.37 C)
6) Implant for Restoring Sexual Potency after PROSTATECTOMY (for prostate cancer patients, see above)

A glance to the future!

I strongly believe that the following applications will become real in the near future

1) **Light based *Brain-Machine mass data* transfer** interface electrodes
2) **Current based *high frequency Brain-Machine mass data transfer*** interface electrodes based on metal or semiconductor nanotubes/fibers
3) Precise optical **diagnosis of epithelial cancer**, based on polarized and backscattering light information
(I suggested a special optical probe)
4) **Vibrating neural implants** that can encourage fast nerve regeneration and repair in nerve-damaged patients
(A fiber based vibrating sample implant has already been made during this master work)
5) Smart implants for cell ***differentiation and Retinal repair*** (Several sample implants have already been made during this master work)
6) **Bio Brain-Machine mass data transfer interface (`I have an idea!`)**

Possibilities are endless!

Appendix 1

Sample staining process

1) Culture medium is removed from the dish.
2) PFA (ParaFormaldehyde) is added.
3) The samples kept in the refrigerator for or one day.

Next day:

The ParaFormaldehyde is removed from the samples.

First Anti body (β-3 Tubulin 1:800 buffer)

1) Buffer of PBS (phosphate-buffered saline) containing 0.25% BSA + 0.25% Triton X is prepared. Then a mixture of β-3 Tubulin (1:800) and the buffer is prepared. (e.g. 1 μl of β-3 Tubulin + 800 µl buffer)
2) Droplets of the prepared solution were dropped on the surface of the sample in a way so that the surface of the fibers and DRGs are covered. (Fig.72)
3) The samples are incubated 24h at 8°C.

Next day:

Secondary Anti body (goat anti-mouse antibody 1:500 buffer)

1) The first antibody is removed from the samples.
2) The samples are rinsed with PBS for 20 min 3 times.
3) The secondary antibody is added to the samples (in a same method that is used for the first antibody) and kept in room temperature for 2 hours.
4) The antibody is removed.

Bisbenzimide (1:1000 PBS)

1) Droplets of bisbenzimide (1:1000 PBS) are added to the samples.
2) The samples are kept in room temperature for 5 min.
3) The bisbenzimide is removed.
4) The samples are rinsed with PBS for 20 min 3 times.

Mounting:

Samples are mounted with PBS Glycerol on a glass slid. (1:1)

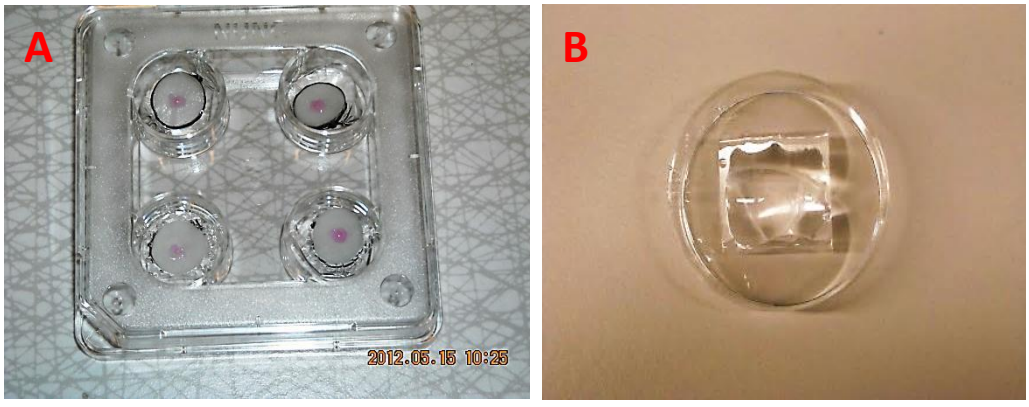


Fig.72: Surface of the samples were covered with a solution of antibody

A) Slices of a microtube assembly with mounted DRG in the center
B) Aligned fibers on a coverslip with mounted DRG in the center

Appendix 2

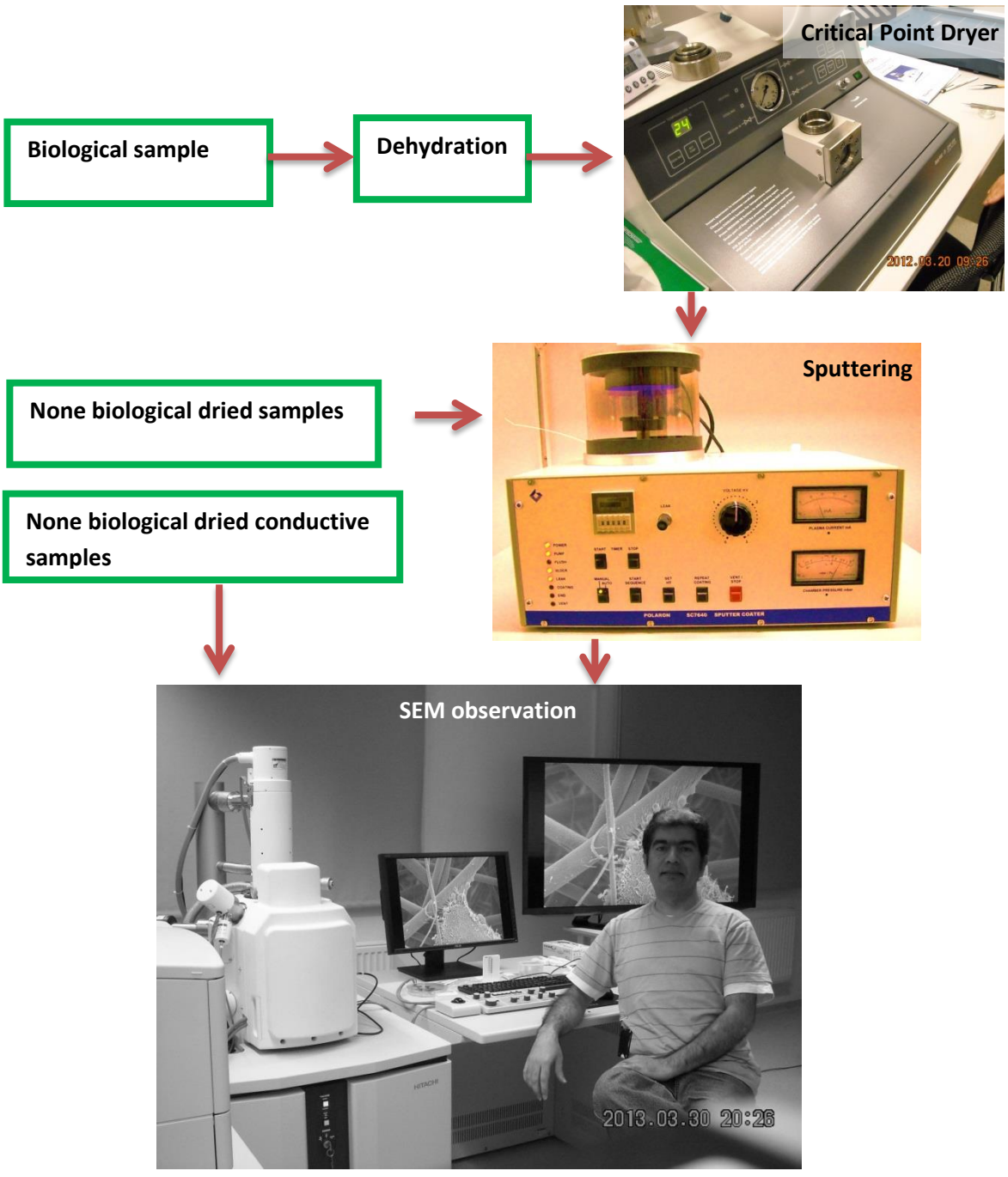
Sample preparation for SEM observation:

Biological sample preparation:

1) Samples are fixed with “Glutaraldehyde 25%” (10% in PBS).
2) After 2 hours the fixative is removed and then the samples are rinsed with PBS 3 times.
3) Gradual dehydration is done with series of ethanol solutions. (50%-70%-95%-100%; 10 minutes each).
4) Samples are dried using Critical Point Drying machine.
5) Sputtering process takes place and samples are coated with a thin layer of Gold- Platinum alloy.

None biological sample preparation:

1) For none conductive samples only sputtering process takes place.
2) Conductive dried samples can be observed in SEM without any preparation.



Appendix 3 3D Images

- **(Red/ blue glasses is required)**

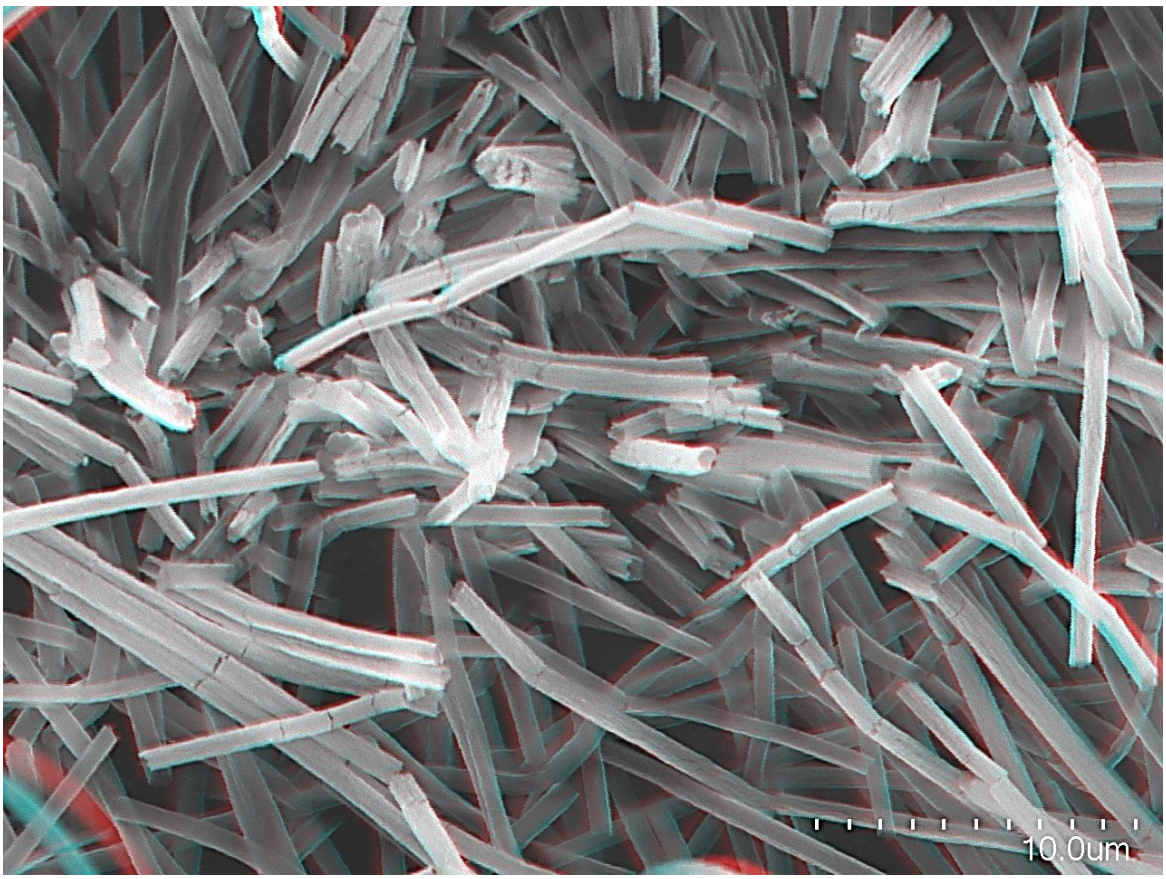


Fig.73 Gold-palladium nanotubes

-

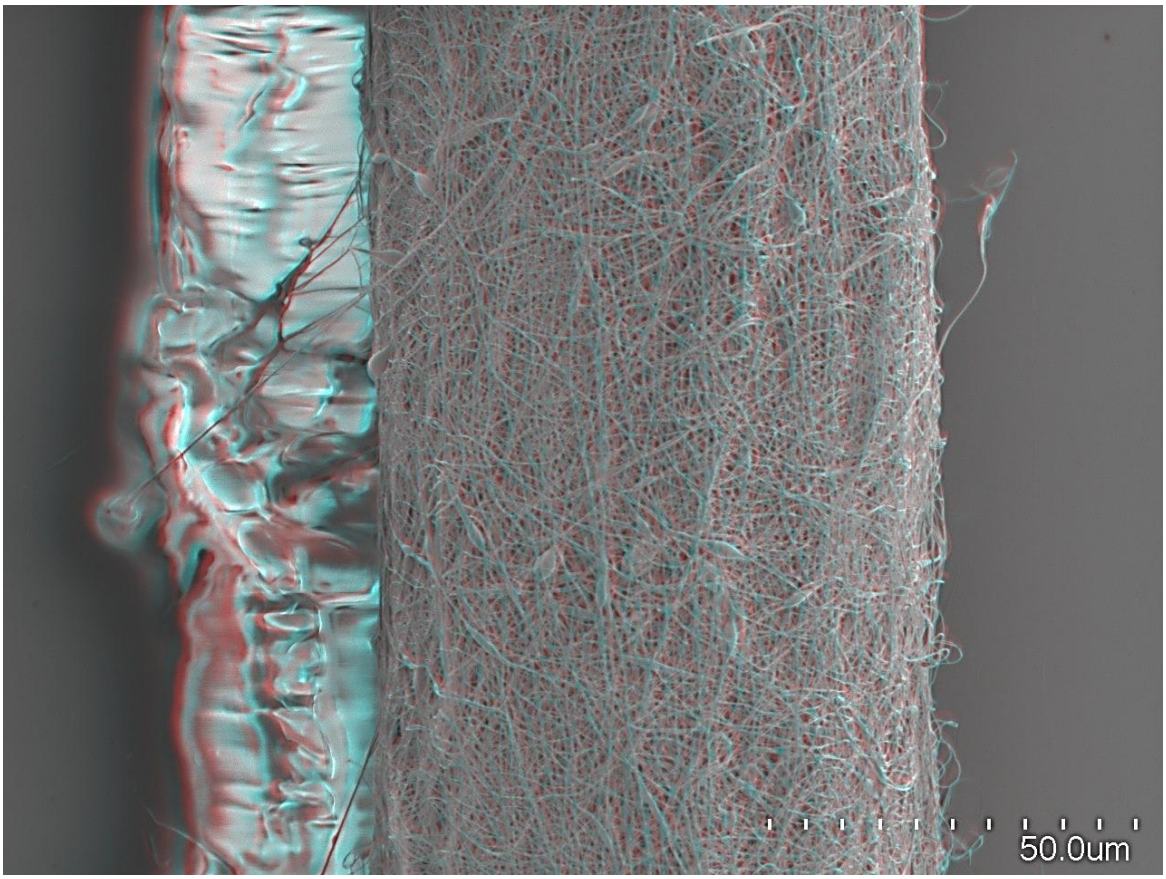


Fig.74. Platinum Iridium micro wire coated with Polyimide nanofibers

Fig.74. Cell-division within the PLLA aligned fibers

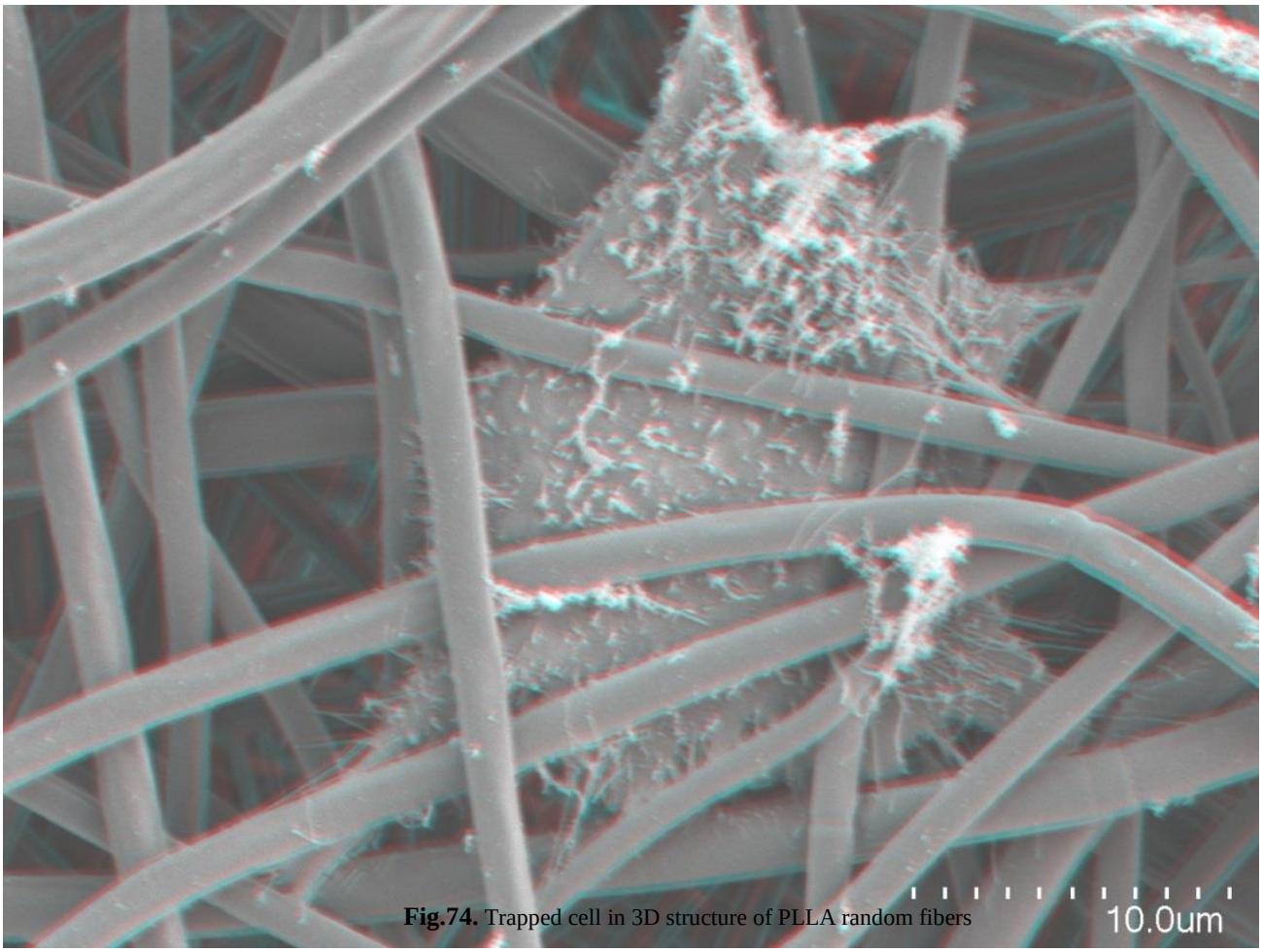


Fig.74. Trapped cell in 3D structure of PLLA random fibers

References:

1) Fundamental “Nano effects” Luisa Filipponi and Sutherland. Interdisciplinary Nanoscience Center (iNANO) Aarhus University, Denmark January 2010

2) Quantum size effects in nanostructures Lecture notes to the course *Organic and Inorganic Nanostructures Kjeld Pedersen* Department of Physics and Nanotechnology Aalborg University

3) DOI: 10.1177/0040517511424524 Textile Research Journal published online 19 October 2011 Rajkishore Nayak, Rajiv Padhye, Illias Louis Kyratzis, Yen Truong and Lyndon Arnold Recent Advances in Nanofibre Fabrication Techniques

4) Polymer Nanofibers Prepared by Template Melt Extrusion Huayi Li,1,2 Yucai Ke,1 Youliang Hu1 1Joint Laboratory of Polymer Science and Materials, Key Laboratory of Engineering Plastics, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China 2Graduate School of the Chinese Academy of Sciences, Beijing, 100039, China Received 28 June 2004; accepted 19 February 2005 Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/app.22597

5) Nanometre diameter fibres of polymer, produced by electrospinning. Darrell H Reneker and Iksoo Chun Maurice Morton Institute of Polymer Science, The University of Akron, Akron, OH 44325-3909, USA Nanotechnology 7 (1996) 216–223. Printed in the UK

6) Recent advances in nanofibre fabrication techniques Rajkishore Nayak, Rajiv Padhye, Illias Louis Kyratzis, Yen Bach Truong and Lyndon Arnold. Textile Research Journal 82(2) 129–147 @ The Author(s) 2011

7) A review on polymer nanofibers by electrospinning and their applications in nanocomposites Zheng-Ming Huanga, Y.-Z. Zhangb, M. Kotakic, S. Ramakrishna Department of Engineering Mechanics, Tongji University, April 2003

8) Electrospinning of Polymers, Their Modeling and Applications Aditya Kulkarni a , V. A. Bambole b & P. A. Mahanwar a a Department of Polymer Engineering and Technology, University Institute of Chemical Technology, Matunga (E), Mumbai b Department of Applied Physics, University Institute of Chemical Technology, Matunga (E), Mumbai Version of record first published: 13 Apr 2010.

9) Electrospinning of Nanofibers Thandavamoorthy Subbiah,1 G. S. Bhat,2 R. W. Tock,1 S. Parameswaran,3 S. S. Ramkumar4 1Department of Chemical Engineering, Texas Tech University; Box 41163, Lubbock, TX 79409-1163 2Materials Science and Engineering, The University of Tennessee, Knoxville 3Mechanical Engineering, Texas Tech University, Box 41163, Lubbock, TX 79409-1163 4The Institute of Environmental and Human Health, Texas Tech University, Box 41163, Lubbock, TX 79409-1163 Received 25 May 2004; accepted 22 June 2004 DOI 10.1002/app.21481 Published online in Wiley InterScience (www.interscience.wiley.com).

10) J Mater Sci (2009) 44:1357–1362 DOI 10.1007/s10853-008-3010-6 The effect of temperature and humidity on electrospinning S. De Vrieze Æ T. Van Camp Æ A. Nelvig Æ B. Hagstro¨m Æ P. Westbroek Æ K. De Clerck

11) Macromolecules 2002, 35, 8456-8466 Micro- and Nanostructured Surface Morphology on Electrospun Polymer Fibers Silke Megelski, Jean S. Stephens, D. Bruce Chase, and John F. Rabolt, Department of Material Science and Engineering and Delaware Biotechnology Institute, University of Delaware, Newark, Delaware 19716; and Central Research and Development, Experimental Station, Dupont, Wilmington, Delaware 19880 Received March 21, 2002

12) Anal. Chem. 2003, 75, 1239-1244 Electrophoretic Protein Transport in Gold Nanotube Membranes Shufang Yu, Sang Bok Lee, and Charles R. Martin Department of Chemistry and Center for Research at the Bio/Nano Interface, University of Florida, Gainesville, Florida 32611-7200

13) Small-scale effect on the mechanical properties of metallic nanotubes Jin Zhang, Chengyuan Wang,a) Rajib Chowdhury, and Sondipon Adhikari College of Engineering, Swansea University, Singleton Park, Swansea, Wales SA2 8PP, United Kingdom (Received 24 June 2012; accepted 14 August 2012; published online 30 August 2012)

14) Immobilisation of glucose oxidase within metallic nanotubes arrays for application to enzyme biosensors Marc Delvaux, Sophie Demoustier-Champagne 1, Unite´ de Chimie et de Physique des Hauts Polyme`res, Universite´ Catholique de Louvain, Place Croix du Sud, 1, B-1348 Louvain-la-Neuve, Belgium Received 4 April 2002; accepted 22 August 2002

15) TISSUE ENGINEERING Volume 11, Number 9/10, 2005 © Mary Ann Liebert, Inc. Fabrication and Endothelialization of Collagen-Blended Biodegradable Polymer Nanofibers: Potential Vascular Graft for Blood Vessel Tissue Engineering WEI HE, M.Sc., THOMAS YONG, Ph.D.,1 WEE EONG TEO, B.Sc., ZUWEI MA, Ph.D. and SEERAM RAMAKRISHNA, Ph.D.

16) Nano Res (2009) 2: 462 473 DOI 10.1007/s12274-009-9042-7 Nanostructured 3-D Collagen/Nanotube Biocomposites for Future Bone Regeneration Scaffolds Edelma E. da Silva, Heloisa H. M. Della Colleta, Andre S. Ferlauto, Roberto L. Moreira, Rodrigo R. Resende, Sergio Oliveira1, Gregory T. Kitten, Rodrigo G.

17) Co-axial Electrospinning for Nanofiber Structures: Preparation and Applications A. K. MOGHE1 AND B. S. GUPTA2 1Doctoral Student, Fiber and Polymer Science and Biomedical Engineering, NC State University 2Department of Textile Engineering, Chemistry and Science, North Carolina State University, Raleigh, NC, USA Polymer Reviews, 48:353–377, 2008 Copyright # Taylor & Francis Group, LLC ISSN 1558-3724 print/1558-3716 online DOI: 10.1080/15583720802022257

18) Bending instability of electrically charged liquid jets of polymer solutions in electrospinning Darrell H. Reneker, Alexander L. Yarin, Hao Fong and Sureeporn Koombhongse

19) http://www.fibercellsystems.com/advantage.htm (Access date Jan 7, 2013)

20) Axon Stretch Growth: The Mechanotransduction of Neuronal Growth Joseph R. Loverde, Rosa E. Tolentino, Bryan J. Pfister1 1Departments of Biomedical Engineering, New Jersey Institute of Technology 2Graduate School of Biomedical Sciences, University of Medicine and Dentistry of New Jersey Correspondence to: Bryan J. Pfister at pfister@njit.edu

21) http://en.wikipedia.org/wiki/Electrical_brain_stimulation (Access date May 8, 2011)

22) Vadim S. Polikov a, Patrick A. Tresco b, William M. Reichert a Response of brain tissue to chronically implanted neural electrodes (Journal of Neuroscience Methods 148 (2005) 1–18)

23) Use of the Skin Effect for Detection of Interconnect Degradation Michael H. Azarian, Daeil Kwon, and Michael Pecht, Center for Advanced Life Cycle Engineering (CALCE) University of Maryland College Park, MD 20742

24) J Comp Neurol. 1991 Dec 1;314(1):125-35. Differential immunochemical markers reveal the normal distribution of brain macrophages and microglia in the developing rat brain. Milligan CE, Cunningham TJ, Levitt P. Source Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia 19129.

25) J Biol Chem. 2009 November 6; 284(45): 31052–31061. Published online 2009 August 27. doi: 10.1074/jbc.M109.052969

PMCID: PMC2781505
Identification of Neuronal Nuclei (NeuN) as Fox-3, a New Member of the Fox-1 Gene Family of Splicing Factors
Kee K. Kim, Robert S. Adelstein, and Sachiyo Kawamoto

26) Neurochemical Research
October 2000, Volume 25, Issue 9-10, pp 1439-1451
Glial Fibrillary Acidic Protein: GFAP-Thirty-One Years (1969–2000)
- Lawrence F. Eng,
- Roopa S. Ghirnikar,
- Yuen L. Lee

27) Deep Brain Stimulation
Drew S. Kern, MS, and Rajeev Kumar, MD, FRCPC - 2007 Lippincott Williams & Wilkins

28) Dopamine D1-D2 receptor heteromer signaling
pathway in the brain: emerging physiological relevance Ahmed Hasbi, Brian F O'Dowd, and Susan R George

29) Textile Research Journal published online 19 October 2011
Rajkishore Nayak, Rajiv Padhye, Illias Louis Kyratzis, Yen Truong and Lyndon Arnold
Recent Advances in Nanofibre Fabrication Techniques . DOI: 10.1177/0040517511424524

30) Electrospinning and Polymer Nanofibers: Process Fundamentals
P K Bhattacharjee, Monash University, Clayton, VIC, Australia
G C Rutledge, Massachusetts Institute of Technology, Cambridge, MA, USA
2011 evier Ltd. All rights reserved.

31) Melt spinning of continuous filaments by cold air attenuation, Jun Jia, School of Materials Science and Engineering-Georgia Institute of Technology, December 2010

32) Introduction to Fiber Spinning and Characterization, Qing Shen, Donghua University, Shanghai, China

33) Electrospinning of Nanofibers from Polymer Solutions and Melts
D.H. Renekera, A.L. Yarinb, c, E. Zussmanb, H. Xua, d
Department of Polymer Science. The University of Akron, Akron, Ohio 44325-3909, USA
Faculty of Mechanical Engineering, Technion – Israel Institute of Technology, Haifa 32000, ISRAEL
Department of Mechanical and Industrial Engineering, University of Illinois at Chicago, Chicago 60607-7022, USA d The Procter & Gamble Company, Winton Hill Business Center, 6280 Center Hill Ave, Cincinnati, Ohio 45224, USA

34) Studies Towards High-Throughput Production of Nanofiber Yarns Studies Towards High-Throughput Production of Nanofiber Yarns by Eugene Smit Dissertation presented for the degree of Doctor of Philosophy (Polymer Science) at the University of Stellenbosch December 2008

35) http://www.nanohole.info/ny.htm (Access date May 5, 2013)

36) Metal Nanofi bers with Highly Tunable Electrical and Magnetic Properties via Highly Loaded Water-Based Electrospinning Nathaniel S. Hansen , Daehwan Cho , and Yong Lak Joo, 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

37) Nanowire electrodes for high-density stimulation and measurement of neural circuits
Jacob T.Robinson, MarselaJorgolli and HongkunPark

38) Biomedical applications of nanofibers Victor Leunga and Frank Koa Wiley Online Library: 29 December 2010

39) Joachim H. Wendorff, Seema Agarwal, Andreas Greiner Electrospinning 2012 Wiley.VCH Verlag & Co. KGaA, Boschstr. 12, 69469 Weinheim, Germany

40) Electrospinning versus fibre production methods: from specifics to technological convergence
C. J. Luo, Simeon D. Stoyanov, E. Stride, E. Pelan and M. Edirisingh