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Fabrication of nano-fibrous silk scaffold for NTE (Neural Tissue Engineering)

M.Tech.Scholar Vipul Kumar
Indian Institute of Technology
Roorkee,
Uttrakhand, India
vipulkumarsinghdocuments@gmail.com

Shalini Priya
Veer Bahadur Singh Purvanchal
University, Jaunpur,
Uttar Pradesh, India

***Abstract* – Tissue Engineering is a promising and emerging area in the field of medical science for treatment of irreparable injuries. It works in synchronization of scaffold and living entity. Silk has favorable characteristics to be used as a scaffold material. Scaffold made up of silk can provide better scope for treatment of neural disorders and injuries through the implications of basic principles of NTE (Neural Tissue Engineering).**

***Keywords* – Nanofiber, Scaffolds Cells, Neural Tissue Engineering.**

I .INTRODUCTION

Tissue engineering is demand of time. It provides an alternative of faster injury treatment with the help of scaffold and some growth factors. We can grow an entire bio-functional organ with the help of tissue engineering. Neural tissue engineering is helpful and provides hope for neural tissue regeneration after the injury. It is a hope for patients suffering from various life threatening diseases like Alzheimer's. Silk is viable and suitable material for construction of scaffold that can be used in Neural Tissue Engineering (NTE). Various unique properties of silk make it a suitable choice for scaffold preparation.

II.REVIEW OF LITERATURE

1. Scaffold- An Integral Part Of Tissue Engineering

Scaffold is basically a framework (3D porous architecture made up of Biomaterials/synthetic polymers) which provides space for living cells to grow in the presence of growth factors. Its fibrous structure provides more surface area for cells to grow and to regenerate. Nano-fibrous scaffolds provide more surface area for cell adhesion and proliferation. Scaffold should be biodegradable. It should be viable for cell functioning and it should not harm functionality of living entity.

It is an integral part of Tissue engineering. Following figure 1, depicts the interaction of scaffold, cells and growth factors which ultimately leads to generation of a bio-functional organ which can be used in case of injury. It provides a hope to those who have lost their vital organs or have lost functionality of any organ due to some accident. Various materials with several modifications have been tried till now. Following table provides details of materials which have been used in NTE

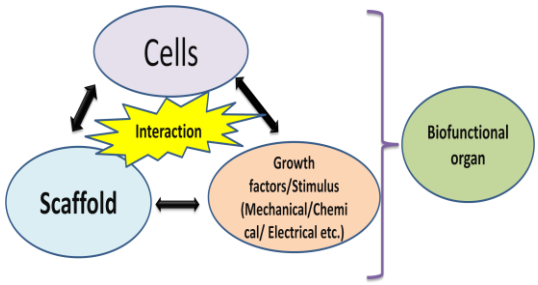

Fig.1 Interaction of Cells, Scaffold And Growth Factors.

2. Which Materials Have Been Used Till Now For Scaffold Fabrication, For NTE?

Table 1. Modified Biomaterials to fabricate scaffold for nerve cell Regeneration

Biomaterials	Modification/Method of fabrication	Improved properties
Star-poly(ethylene glycol)	incorporation of polysachcharides heparin	tunable physical and mechanical properties
Collagen	Hydrogel croslinked with peptide modified dendrimers	Biological functions
Poly (glycerol-sebacate)	Replica molding	Flexibility,surface degradable
Chitosan	Poly lysine functionalized thermo responsive chitosan hydrogels	mechano-compatibility
Poly lactic co-glycloic acid	low pressure injection molding	porosity

3. What are the limitations of material which have been used in NTE?

Till now scaffold of various material have been fabricated for application in neural tissue engineering but none of them provides:-

- complete non-immunogenic response,
- controlled degradation,
- desired mechanical strength,
- biodegradability etc.

4.Can silk be used as an ideal material for scaffold preparation in NTE?

Silk has all the favorable qualities to be used as scaffold material for NTE. Silk is naturally occurring material and it can easily be harvested from silk cocoon. It has high mechanical strength. It can be woven into a mesh like structure which can further be used as a scaffold. Its fibrous structure provides porosity to scaffold which is a crucial factor in tissue engineering. Silk's degradation can be controlled with its modification. It can be turned into a solution like form which can later be electrospun in the form of scaffold. Following figure describes the importance of silk as a tissue engineering material.

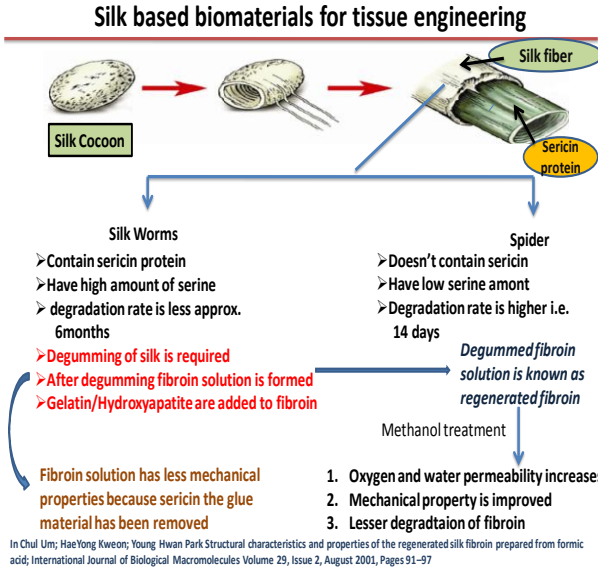

Fig.2 Silk as a Biomaterial for Tissue Engineering**.**

III. OBJECTIVE

My aim is to fabricate a scaffold with single natural material having similar properties as that of ECM (Extra Cellular Matrix) and all other desired Mechanical & Chemical properties.

IV. METHODOLOGY

Silk cocoons are a good source of silk fibers. Silk cocoons are basically egg like structure inside which insect lives. First of all we will have to dry the silk cocoon and then only insects can be removed out after cutting the cocoon. With the treatment of cocoon with various chemicals; silk solution can be obtained. Protocol for preparation of silk solution has been provided in figure below.

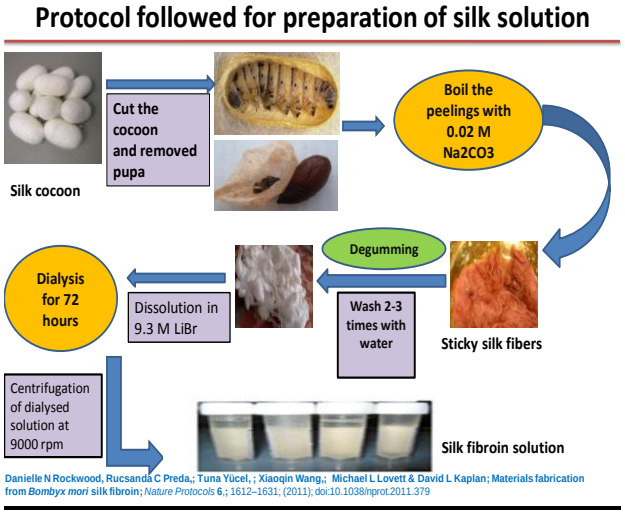

Fig. 3 Steps for preparation of silk solution from silk Cocoon.

After preparation of silk solution, it's time to prepare scaffold. There are Several Techniques available for Scaffold Preparation. Names of techniques available are given below:-

1. Nano fiber self assembly
2. Solvent casting and particulate leaching
3. Gas foaming
4. Emulsification/ freeze drying
5. Liquid-liquid phase separation
6. Electro-spinning

Out of these techniques we chose Electro-spinning, because it has following advantages:-

1. Provides control over morphology porosity and composition using simple equipment.
2. Variety of fibers can be used in the process.
3. Nano-fibers with of 40-2000 nm can be produced by selecting suitable combination of polymer and solvent to be used.

Basic principle of Electro-spinning has been given in figure below:-

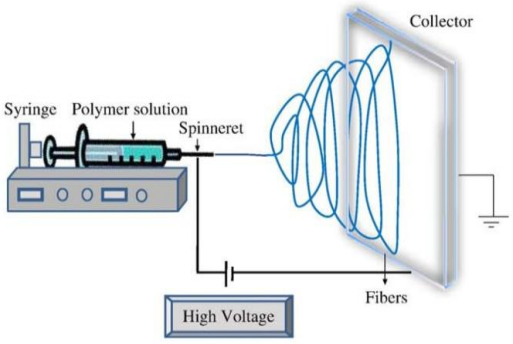

Fig.4 Principle of Electro-Spinning

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Fig. 5 Optimization of electro-spinning parameters for fabrication of silk nano-fibers**.**

There are various factors which affects morphology of silk fibers produced through electro-spinning. There are basically4 electro-spinning factors affecting morphology of silk fibers generated from Bombyx mori silk coccoon. These factors are as following:-

- Electric field.
- Tip-to-collection plate distances.
- Silk concentrations.
- Flow Rate.

Now there is need to optimize these factors for successful production of silk fibers for scaffold preparation. These factors were optimized through various hits and trials.

Figure given below shows optimization of various factors with various conclusions in concise form.

Optimized parameters found were as following:-

- Concentration=10%
- Distance=12 cm
- Voltage= 15KV
- Flow rate= 2.3 ml/hour

We applied these parameters and tried to electrospin silk solution and collected it on a metal mesh. Later on we observed the work under various magnifications. Details are being given in figure below.

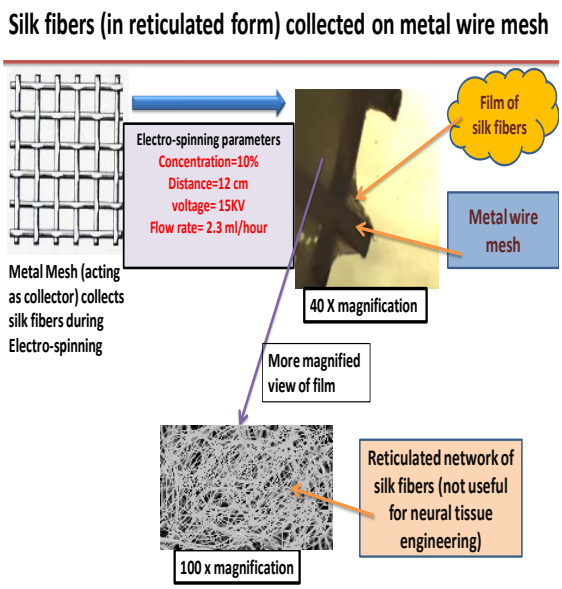

Fig. 6 Silk fibers (in reticulated form) collected on metal wire mesh.

There were various problems associated with reticulated network of fibers. Main problem was associated with proliferation and differentiation of neural cells because of the following reasons:-

These problems were solved using rotating collector. Figure showing working of rotating collector is given below

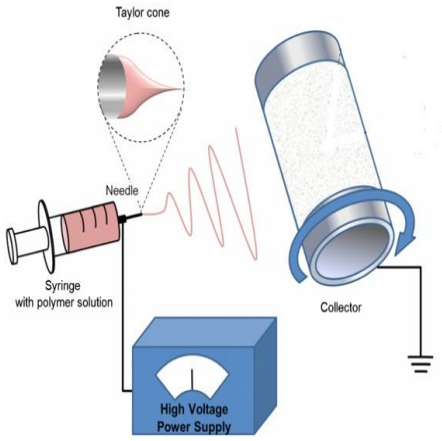

Fig.7 Rotating Collector in Electro-spinning for Scaffold preparation.

Viscosity of solution is also important for electro-spinning. If viscosity is not proper then fibers can't be produced. Proper viscosity can be obtained only and only if suitable solvent is used for preparing silk solution from fluffy mass of silk fibers. Organic solvents were found suitable for producing optimum viscous solution.

Following figure 8 depicts the need of optimum solvent for a successful silk scaffold preparation.

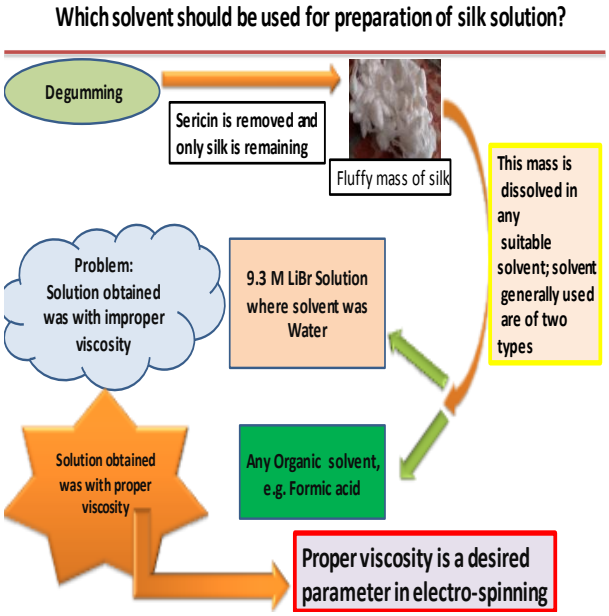

Fig. 8 Selection of appropriate solvent for producing solution of proper viscosity.

After optimization of all factors of electro-spinning and using rotating collector, we were finally able to obtain a scaffold with parallel fiber orientation. Parallel fibers provide suitable environment to neural cells for proliferation and differentiation.

Parallel fibers obtained were observed under 100X magnification. Following figure 9 shows the fibers obtained with all the concluded parameters of electro-spinning.

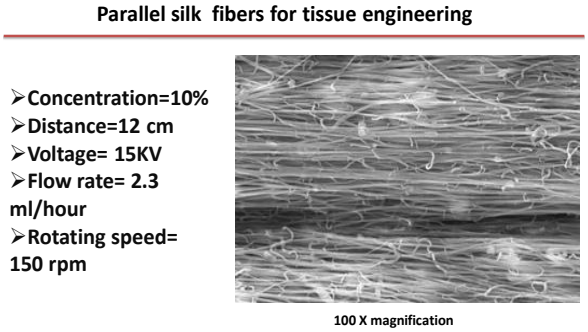

Fig. 9 Parallel fibers obtained after electro-spinning of silk solution with all the optimized parameters.

V.CONCLUSION

By using following parameters parallel silk fibers can be obtained which can be effectively used for neural tissue engineering. Process parameters for electro-spinning of silk solution:-

- Concentration=10%.
- Tip Target Distance=12 cm.
- Voltage Applied= 15 KV.
- Flow rate of silk solution= 2.3 ml/hour.
- Rotating speed of collector= 150 rpm.

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