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ALIGNED ELECTROSPUN PLGA NANOFIBERS AS FUTURE NERVE CONDUIT

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SUMMARY

The study's objective was to develop aligned biocompatible scaffold human nerve conduit for future use in peripheral nerve regeneration. Collagen matt was prepared using ovine collagen and cross-linked with 0.1% genipin. PLGA nanofibers were fabricated using electrospinning onto the collagen matt on a rotating mandrel with specific optimized parameter to achieve 700-1000nm fibers with 70-80% unidirectional alignment. The results showed that neural-differentiated mesenchymal stem cells could adhere to the nanofibers and remain viable and also proliferate while maintaining its respective marker such as Nestin, S100 β , p75 NGF and GFAP. The collagen matt together with cell-seeded nanofibers was rolled into a tubular nerve conduit which may be ideal to facilitate

1.0 Introduction

Peripheral nerve regeneration has been gaining much attention in the field of study due to the conventional method and functional recovery has often been incomplete or unsuccessful. This in turns will affect the patients' life quality as neuropathy pain is always the complication of peripheral nerve injury (PNI)^{1,2}. Nerve autograft is the gold standard in most surgical nerve cases to bridge gap between nerves in which sural nerve taken from the injured patient will be commonly used^{3,4}.

Allografts is an alternative for nerve regeneration however there's a major concern on immunogenic reactions in the recipient, in which requires the recipient to be immunosuppressed until the host Schwann cells regenerated on the nerve graft⁵. Hence, the usage of electrospinning technique to produce a variety of nerve conduit with various advantages such as aligned fibers, tunable porosity, and malleability can be used as an alternative future nerve conduit^{6,7}.

2.0 Materials and Method

2.1 Collagen Sheet Fabrication

Collagen was obtained from Ovine tendon and soaked with 0.35M acetic acid for 3 days before blending it into a solution form. NaCl salt was added to precipitate the collagen and undergo dialysis using dialysis tubing made of cellulose membrane of 14kD MWCO to remove excess acetic acid for 3 days. The collagen solution was then freeze-dried to remove the solvent and later be dissolved back into 0.35M acetic acid in a concentration of 15mg/mL. The solution was then poured into a square casting before freeze-dry to produce a sheet of collagen and cross-linked with 0.1% genipin.

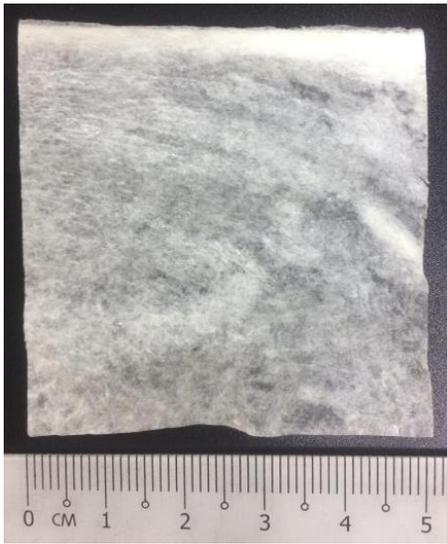


Fig. 1 Fabricated collagen mat

2.2 Electrospinning

A weight of 1.0g of PLGA 85:15 was dissolved in 5ml of DCM:DMF mixture (4:1) to produce 20% concentration of PLGA solution and mixed on a shaker overnight. The polymer solution was then electrospun on top of the collagen sheet with the following parameter. The nanofibers with the collagen sheet were then UV for 30 minutes for sterilization before cell seeding.

Table 1. Electrospinning parameters

Distance (cm)	Voltage (kV)	Flow rate (ml h ⁻¹)	Rotating Mandrel Speed (rpm)
33	12-14	0.15	1400

2.3 Cell Culture and Seeding

Bone marrow mesenchymal stem cell (BM-MSC) was cultured using alpha minimum essential medium (α -MEM) with 10% FBS and 1% antibiotic until P3. A series of concoction with the following chemicals and growth factors in

the α -MEM was then used to induce the BM-MSCs into neural-like cells. Neural protein markers (p75 NGF, S100 β , Nestin and GFAP) were then detected via immunocytochemistry.

Table 2 Neural induction mediums for the BM-MSCs

Days of incubation	Chemical/Growth Factor	Concentration in medium
1	β -Mercaptoethanol	1mM
3	FBS	10%
	All Trans Retinoic Acid (ATRA)	35ng/mL
5	FBS	10%
	bFGF	10ng/mL
	PDGF-AA	5ng/mL
	Heregulin	200ng/mL
	Forskolin	5 μ M
	B ₂₇	2%

The induced MSCs were then seeded onto the PLGA nanofibers at a seeding density of 3000 cells/cm² in a 12-well plate. The seeded cells were cultured for another 7 days followed by detection of neural protein markers expression via immunocytochemistry.

2.4 Fiber Characterization

The electrospun nanofibers were evaluated using Scanning Electron Microscope (FEI, USA). Diameter of the fibers and the alignment of the fibers were determined using Image J software (NIH, USA).

3 Results

3.1 Fiber Characterization

Most of the nanofibers appeared smooth and aligned. When sampled from 200 fibers were measured, approximately 80% were aligned in one direction and has a diameter ranging from 700-1000nm. Figure 2 shows the morphology of the fibers spun on a collagen sheet seen under the SEM.

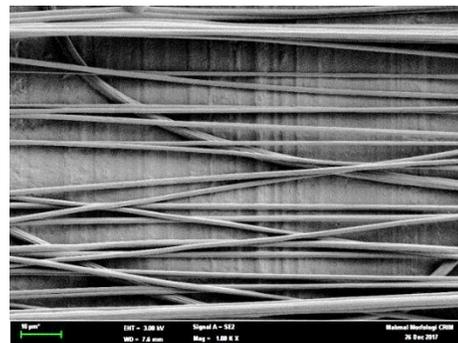


Fig. 2 Nanofibers under SEM imaging (1000x magnification)

3.2 Neural Marker Expression

The induced MSCs were subjected to immunocytochemical staining to detect the protein expression of GFAP, S100 β , Nestin and p75 NGF.

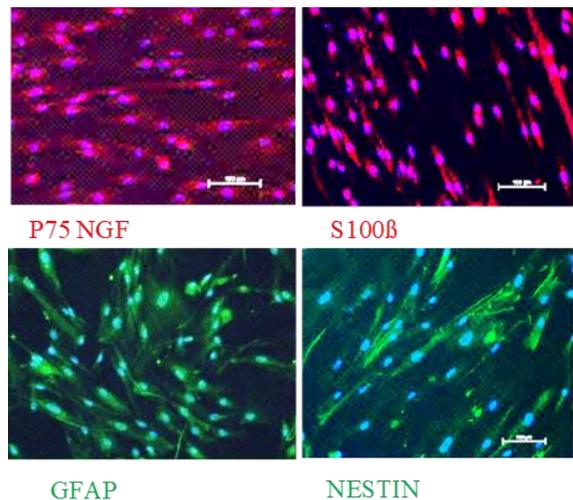


Fig. 3 Protein expression of p75 NGF (red), S100 β (red), GFAP (green) and Nestin (green) by neural-differentiated MSCs seeded for 7 days on PLGA nanofibers. Cell nucleus were counterstained with DAPI (blue). Magnification: 40X (Scale bar: 100 μ m)

4.0 Discussion & Conclusion

We have successfully electrospun PLGA nanofibers with unidirectional alignment. This is deemed crucial as nerve fibers in peripheral nerves are also unidirectional. BM-MSCs were successfully induced into neural-like cells as demonstrated by expression of neural protein markers such as nestin, S100 β , GFAP, and p75 NGF. The expression of the neural markers persisted after the seeding of these cells on the PLGA nanofibers for 7 days. In our observation, the cells attached preferentially on the nanofibers compared to the collagen mat, and they migrated along the nanofibers in a unidirectional manner. This attribute greatly supports the use of these nanofibers as nerve conduit to facilitate the migration of neural cells from the proximal stump of the peripheral nerve during axonal regeneration. The collagen mat used to support the nanofibers can be rolled into a cylindrical conduit to bridge a peripheral nerve gap. Furthermore, the collagen mat provided the mechanical strength required for the conduit to be sutured onto the peripheral nerve tissue. Following this success, proof of concept in animal models will be performed.

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