BT5130 Tissue Engineering

Neural Tissue Engineering: A detailed review of the recent developments

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Introduction

The central nervous system in mammals consists of the brain and the spinal cord, including the blood-brain barrier that restricts different kinds of molecules that reach these organs. The ability of this system to self-heal and regenerate is very minimal [1]. Hence, neuro-injuries and Spinal Cord Injuries pose high severity and challenges. CNS injuries can be apoptotic and necrotic death of neurons, astrocytes and oligodendrocytes, axonal injury, ischemia, and inflammation [2]. The extracellular matrix (ECM) can also get severely affected at the site of damage. These challenges, therefore require a mechanism to support neurogenesis in case of injury, while also ensuring that the side effects are not persistent. Autologous grafts are highly advantageous; however, they might cause morbidity at the site of insertion, and the supply is, of course, limited [2]. Allogeneic and xenogeneic grafts pose high risks of immune rejection. The intricacies in a neural tissue demand a successful construct that incorporates multiple features in tissue engineering and provides a cooperative environment for neurogenesis and healing. This review will briefly explain the recent developments in the different kinds of biomaterials, cells and stimuli used in neural tissue engineering. I will also mention the current challenges that need to be addressed and propose a potentially novel combination of the parts to address the existing problems.

Biomaterials and Scaffolds used in the field

Hydrogel systems:

Hydrogels provide high flexibility and ease in modifying material characteristics to suit neural regenerative requirements. A very common hydrogel tested both *in vitro* and *in vivo* is made of the natural polymer Hyaluronic Acid. Different cells such as NPCs from forebrain cortical neuroepithelium of E13.5 rats, ReNcells and NPCs have all been tested for *in vitro* biocompatibility in HA hydrogels [3].

A detailed analysis elaborating the properties and advantages of HA hydrogels, both *in vivo* and *in vitro* is reported here [4]. Hydrogels systems tend to support neurite growth by providing physical guidance. Madhusudhanan *et al* [5] present an extensive review of the different kinds of property elicited by the hydrogel. In addition to providing mechanical support for neurogenesis, hydrogel systems also aid in the release of drugs and other growth factors. Injectable hydrogels [6] and cryogels gellate after entering the host system [7], and they don't elicit any adverse effect on the cell [8]. Laminin [9] is another natural polymer that has been used to make thermoresponsive hydrogels that may provide a robust delivery vehicle to injured CNS tissue. In general, hydrogels are favourable for their porous structures, biodegradability, non-antigenicity and chelating properties [2] [10]. Despite the noted advantages of biocompatibility, there is still a long way from translation. The restoration of functional connectivity is often very challenging, and one must also look into being able to enhance the pro-regeneration environment within the patient for better effects [5].

Electrospun scaffolds:

Electrospinning offers many advantages for the fabrication of the construct as we have direct control over the physical, chemical and mechanical properties [11]. The architecture can be fine-tuneable, and highly porous design of the construct will aid in easy flow of nutrients and other macromolecules for neural growth and development [12]. Nanofibrous conduit comprising PCL/PLGA polymers are said to promote nerve regeneration across a 10 mm nerve gap in rat sciatic nerve [13]. The differentiation behaviour of different stem cells depends on the fibre orientation and diameter of electrospun scaffolds [14]. Various biofunctionalization strategies for electrospun constructs are elaborately explained in the review by Chen *et al* [15]. Although spider silk was tested for neural growth construct [16], it was not until last year, where a construct composed of spidroin and platelet-rich plasma was successfully implanted in the brain and spinal cord of *Rhesus Macaque* [17]. However, the delivery of electrospun scaffolds *in vivo* is still a primary challenge. One way to circumvent this is to create a hybrid material consisting of an injectable hydrogel containing discontinuous or short electrospun fibres that will

self-assemble *in vivo* based on environmental cues [18]. Different 3-dimensional neural tissue mimics have been constructed and they show spontaneous electrical activity and spatial connectivity [19].

Commercially available constructs:

A few commercially sold and FDA-approved nerve conduits are listed below [20].

Construct	Material	Characteristics	Manufacturer
NeuroTube	Polyglycolic acid	Absorbable woven mesh tube of length 2-4 cm; degrades in 3 months	Synovis Micro companies (FDA approved in 1999)
NeuraGen	Collagen type I	Resorbable implant for peripheral nerve discontinuities; degrades in 3-4 months	Integra LifeSciences Co. (FDA approved in 2001)
Neurolac	Poly-dl-lactide-caprolactone	Tubular construct of length 3 cm; degrades in 16 months	Polyganics BV (FDA approved in 2005)
Neuragen 3D	Type I collagen and glycosaminoglycan (chondroitin-6-sulphate)	Flexible tube of length 6-6.5 cm; degrades in 9-12 months	Integra LifeSciences Co. (FDA approved in 2014)
Nerbridge	Polyglycolic acid and Type I and III collagen	Flexible tubular filled with porous collagen; degrades in 3-4 months	Toyobo Co., Ltd. (FDA approved in 2016)

Types of Cells and Factors that induce cell differentiation used in Neural Tissue Engineering:

The common types of cells used in Neural Tissue Engineering include Schwann cells (SCs), Mesenchymal stem cells from various sources (BM-MSCs, AT-MSCs, hUMSCs), and other stem cells such as embryonic stem cells (ESCs) and Nerve stem cells (NSCs) [2]. **Schwann cells** inside the brain produce neurotrophic factors that induce neurite and axonal outgrowth in case of an injury. Endogenous SCs are attracted by the GDNF particles, increasing the rate of nerve regeneration [21]. However, this approach is almost obsolete because SCs are difficult to purify and can cause immune reactions. Experimental data provided by Rodrigues *et al* show that the immune response caused by the Schwann cells affected the reinnervation process [22]. **Mesenchymal cells** have immunomodulatory properties, produce various growth factors and also differentiate into multiple cell lineages, including neurons and glia. **Bone marrow-derived MSCs** (BM-MSCs) can *in vitro* differentiate into Schwann-like cells and help in axonal regeneration in case of an injury, but the proliferation potential is not sufficient [2].

Adipose tissue-derived MSCs (AT-MSCs) support neurite growth and express a high level of synaptic proteins, as evidence of synapse occurring in the cells. Additionally, AT-MSCs express other vital neurotrophic factors required for proliferation and perform better compared to different kinds of MSCs [23]. **Umbilical cord MSCs** are easily accessible and more immature than other MSCs, and a study shows that hUMSCs can differentiate into cells from all three germ layers. Therefore, differentiation of cells from neonatal tissues into neurons will be more straightforward and efficient [24]. Serum-free culturing conditions have been elaborated for human cord blood stem cells by Hamad *et al* [25]. The inducing signals for differentiation, are provided by Edaravone [26] and Resveratrol [27]. **Embryonic stem cells (ESCs) and Induced pluripotent stem cells (iPSCs)** are known for their ability to self-renew and pluripotency [28]. Hence, any desired cell state can easily be achieved when compared to the other approaches. However, one must always keep in mind the differences between ESCs of model organisms and the host organism to ensure safe transplantation [29]. A detailed review of different ESCs and iPSCs-based therapies for various neuro disorders is given by M Willerth [28]. **Nerve Stem Cells (NSCs)** are what the other stem cells are finally differentiated into, as these cells set out to replace the damaged neurons/ astrocytes/ glial cells under physiological conditions. N, N-dimethyltryptamine (DMT) activates the subgranular neurogenic niche which in turn regulates the proliferation of NSCs, the migration of neuroblasts, and promoting of neurogenesis, thereby improving spatial learning and memory tasks [30]. Other common inducing factors that help in differentiation of the above-discussed cells include a variety of miRNAs [31], Sovateltide (IRL-1620) [32], erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF), retinoic acid, recombinant human bFGF, and culturing of cells at physiological levels of electrolyte for electrical activity [2] [33].

Conclusion:

A commonly observed trait is that there is no one metric to compare different strategies and approaches. One such comparison can be based on the quantification of adult neurogenesis and its performance in the presence of implants [34]. Lesser *in vivo* studies have been conducted, and hence, not all

experiments reached clinical trials. Several research in the field of Neural tissue engineering has been carried out within our nation. This is very briefly explained in this review article by Haldar *et al* [35]. Out of all the strategies that we have discussed in this review, I will henceforth focus on two potential approaches that individually promise safer engineering of neural tissues. Electrospun scaffolds made of **spidroin** are highly biocompatible and are also advantageous for aiding in the nutrient supply for cells. The architecture can also be precisely engineered such that the delivery is supported by injectable hydrogels. **AT-MSCs and hUMSCs** are two kinds of stem cells that are well-studied for neurological purposes, and their combination with spidroin constructs have not been delved into yet. Thus, while each approach shows promising results, combinations of these two strategies may lead to a more successful recovery in the CNS regeneration.

Graphical summary:

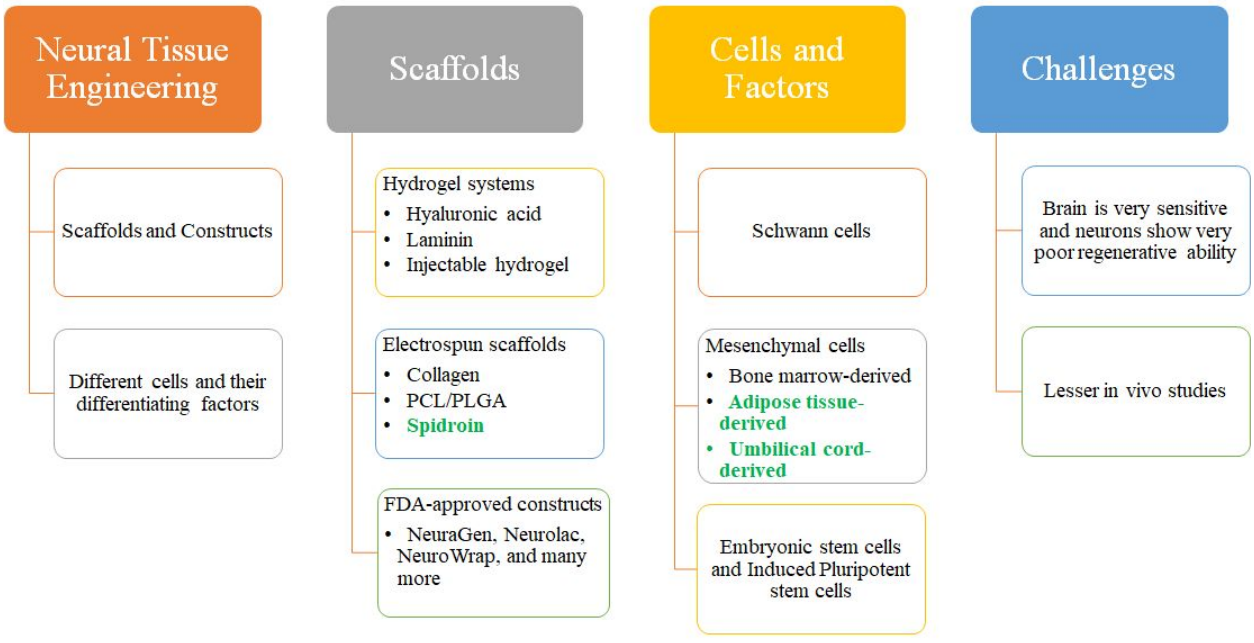

Figure 1: A summary of the review discussed above. The strategies highlighted in green will further be explored in the upcoming reports.

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