Prospects of Natural Polymeric Scaffolds in Peripheral Nerve Tissue-Regeneration

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Abstract

Tissue-engineering is emerging field and can be considered as a novel therapeutic intervention in nerve tissue-regeneration. The various pitfalls associated with the use of autografts in nerve-regeneration after injuries have inspired researchers to explore the possibilities using various natural polymers. In this context, the present chapter summarizes the advances of the various types of natural polymeric scaffolds such as fibrous scaffolds, porous scaffolds, and hydrogels in nerve-regeneration and repair process. The functionalization of the scaffolds with wide-range of biomolecules and their biocompatibility analysis by employing various cells (e.g., mesenchymal, neural progenitor stem cells) along with the *in vivo* regeneration outcomes achieved upon implantation are discussed here. Besides, the various avenues that have been explored so far in nerve tissue-engineering, the use of the extracellular matrix in enhancing the functional polymeric scaffolds and their corresponding outcomes of regeneration are mentioned. We conclude with the present challenges and prospects of efficient exploration of natural polymeric scaffolds in the future to overcome the problems of nerve-regeneration associated with various nerve injuries and neurodegenerative disorders.

Keywords

Nerve tissue-engineering · Natural polymers · Scaffolds · Stem cells · Extracellular matrix

27.1 Introduction

27.1.1 Micro-Architecture of Nerve Cell

The nervous system is the network of nerves responsible for the control, communication, and coordination in the body. Basically, it is divided into two parts, the peripheral nervous system (PNS) and the central nervous system (CNS). The CNS includes the brain and spinal cord and the PNS constitutes the nerves and ganglia. These acts as a communication link between the whole body and the CNS. Neurons are the functional unit of the nervous system and are electrically excitable and terminally differentiated cells. It comprises of the cell body, neuronal axons, the

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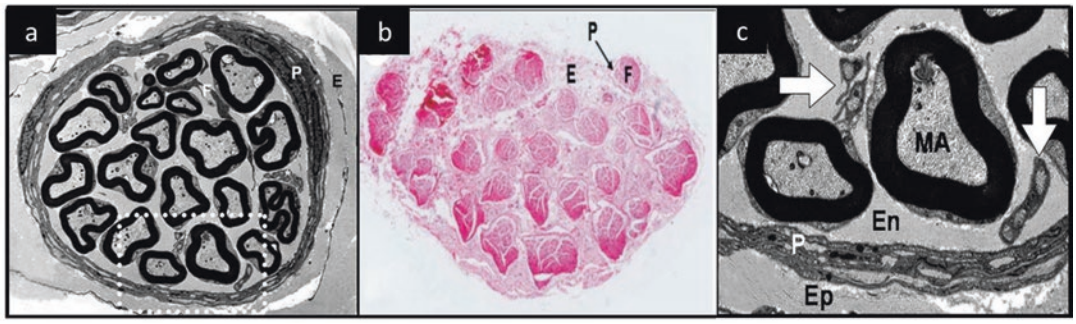

Fig. 27.1 Electron and light microscopy image of the nerve fascicle (F) at (**a** and **b**). The (**c**) highlights the microstructure of nerve showing three layers endoneurium (E/En), perineurium (P), and epineurium (Ep) around myelinated axons (MA). The white arrows represent the non-myelinated neurons. (Reprinted with permission from Elsevier [88])

cells along with connective tissue stroma and a blood supply [88]. In comparison to CNS, the nerve cells of the PNS have some power of regeneration. The cell bodies of PNS neurons are located within spinal ganglia, their central connections (nerve roots) and axons outstretch through peripheral nerves extending to target organs. PNS contain several types of nerves (e.g., afferent sensory nerves and efferent motor nerves) performing the coordination and functional activities [115]. Each nerve is marked out from the surrounding by a protective layer called epineurium (Fig. 27.1). It consists of loose connective tissue and blood vessels that supply the nerve. Within the epineurium, the nerve fibers are contained and aligned in fascicles. Each fascicle is marked out from the other fascicle by a layer called perineurium. It constitutes of dense perineurial cells and collagen fibers which mark its outer layer. Nerve fibers in the fascicle are supported by a connective tissue in their surrounding called endoneurium. Reticular fibers, fibroblasts, collagen, extracellular matrix (ECM) constitute endoneurium [57]. Besides this, alignment of collagen fibrils around the nerve fibers gives rise to endoneurial tubes. The axons along with Schwann cells (SCs) are enclosed in these tubes. These endoneurial tubules provide the efficacious environment for SCs to proliferate and subsequent regeneration and reinnervation after an injury [124].

27.1.2 Importance of Schwann Cells in Nerve-Regeneration

Following injuries, only the nerve cells of the PNS have the power of regeneration and reinnervation. Therefore, permitting some degree of restoration of lost functions, depending upon the graveness of the injury and the quality of the repair in the PNS [4]. Besides, the change at the cellular and molecular levels in Wallerian degeneration, the internal natural ability of the injured neurons to regenerate equally contributes to nerve repair. SCs that are mainly responsible for the myelination of axons play a significant role in the regeneration of nerve cells. Distal SCs at the site of injury switches and dedicate themselves to nerve repair because of their intrinsic flexible different properties that are subdued in oligodendrocytes and neurons of the CNS. SCs at the site of injury proliferate and secrete trophic factors that guide the regeneration process of axotomized neurons [136]. These SCs align themselves along the basal lamina that leads to the formation of the bands of Büngner which offer cues to nerve fiber regeneration. The SCs along with their produced basal lamina (i.e., ECM) and these bands enhance and promote the guided nerve tissue-regeneration by providing the mandatory cellular and molecular factors, including the growth factors [49]. Thus SCs, besides the participation in myelination of axons also participate in guided nerve

tissue-regeneration after an event of nerve injuries. Later on, the recovering neurons will have the ability to reach the distal target organs and subsequent reinnervation restores the lost functions [80]. After peripheral nerve transection, the surgical repair is necessary, to permit the injured axons to grow into the distal degenerating nerve. [42]. This intervention reunites the two nerve stumps proximal and distal. However, this union being difficult, often misguide the axons towards the wrong distal path. Thus, the intervention of autologous grafts is commonly used to overcome this problem [42]. Autografts as the union between the stumps are most commonly used and have remained a gold standard for larger nerve defects that otherwise are difficult to repair. However, their clinical utilization implicates serious drawbacks like lack of donors, donor site morbidity, differences in nerve architecture and SC phenotype mismatch [39, 42].

27.1.3 ECM in Nerve Tissue-Regeneration

ECM of the nervous tissue is comprised of various proteins, which include major fibrous proteins collagen, laminin, fibronectin, fibrin [122]. Besides these proteins nidogens, vitronectin, and carbohydrate polymers covalently bound to proteins called proteoglycans are also present. These include heparin, chondroitin, keratin, dermatan and their sulfates [73]. These ECM fibrous components, especially collagen, laminin, fibronectin play an important role in the nerve-regeneration and hence are preferred in nerve tissue-engineering [57]. Nerve fibers in PNS are contained and aligned in the endoneurium as already mentioned above, where SC gets involved in the process of myelination of axons. Besides, their role in myelination of axons they also produce basal lamina in endoneurium. Basal lamina (i.e., ECM) is composed of collagen IV, laminin and fibronectin [16, 25, 113].These basal lamina layers act as fibrous scaffolds and subsequent proliferation of SCs give existence to bands termed as bands of Büngner. Remembering the role of SCs and basal lamina contained in the endoneurium especially proteins, various approaches have been explored by tissue-engineering towards the reconstruction of nerve gaps after nerve injuries. Instead, of implantation of grafts nowadays tissue-engineering has achieved new heights in regenerative medicine. Moreover, the nerve tissue-engineering has also been studied greatly and engineered nerve tissue grafts are emerging as an encouraging approach against conventional grafts [61, 174, 175].

27.1.4 Tissue-Engineering

Tissue-engineering involves the fabrication of various biocompatible scaffolds for use in *in vivo* transplantation, to replace, repair and/or to regenerate damaged tissue [91]. Tissue-engineering has opened new opportunities and ways towards tissue-regeneration including nerve tissue-regeneration, thus paving a way to overcome the limitations of traditional routes for tissue-engineering like autologous grafts. Polymeric scaffolds of biopolymers are extensively used in the tissue engineering, as fibrous architecture and nanotopographies present in these scaffolds is similar to the ECM present in endoneurium in micro-structural nerve cell anatomy of PNS [9, 31, 128]. Thus, after an event of injury, these scaffolds are capable of guiding the regeneration process across injured lesions.

27.1.5 Fabrication and Functionalization of the Scaffolds for Nerve Tissue-Regeneration

Various polymeric scaffolds for nerve tissue-engineering have been fabricated for use in nerve-regeneration. There are a number of approaches that can be utilized for their fabrication like electrospinning [45], phase separation [110], self-assembly [101], templating [154], drawing [79], vapor-phase separation [139], controlled solution synthesis [78], chemical oxidative polymerization

[40], bacterial cellulose synthesis [133] and extraction from plants [98]. Among these methods, electrospinning technique is the widely used strategy because of its simple, governable processing parameters and fiber functionalization. Due to its versatility, this technique is also widely explored for fabrication of polymeric scaffolds in nerve tissue-engineering [17, 18, 38]. Electrospinning involves the fabrication of polymeric nanofibers from a liquid solution under the high electric field in kilovolts supplied by high voltage power supplier. The polymeric droplet when exposed to a high electric field gets a surface positive charge, resulting in a Taylor cone generation. Subsequently, this droplet is driven in the form of a fiber having diameters at nano-scale depositing towards the grounded collector plate [18]. The fabrication of the scaffolds can be modulated by various parameters like viscosity, conductivity, flow-rate, and tip to collector distance [18]. These parameters were simple and easily tunable providing the versatility of this technique. However, the fabrication of fibers with electrospinning mainly produces scaffolds with 2D architecture. The influence on electrospun 2D polymeric scaffolds during *in vitro* experiments with various types of cells has been extensively studied and certain drawbacks like size limitations, low porosity, low thickness and reduced penetration in the scaffolds came forward [22].

Biomaterial-based polymeric scaffolds are frequently exploited in various nerve tissue-regeneration applications. A huge research has been performed and is currently going on towards the fabrication and functionalization of various scaffolds. Blending with proteins and growth factors has made good progress in tissue-engineering, but challenges of technical and ethical issues are of common concern [26] [164]. The role of ECM in nerve repair after injury has been aforementioned keeping this importance in consideration various ECM functionalized scaffolds have gained ample significance in recent years. The use of ECM-based polymeric scaffold in nerve tissue-regeneration has thus reduced the drawbacks associated with cells or growth factor functionalized scaffolds [174].

27.1.6 Natural Polymeric Scaffolds in Nerve Tissue-Engineering

Neural scaffolds composed of various biomaterials have been engineered and are providing new hopes to treat various neurodegenerative disorders [122]. Studies have shown that ECM components and biomimetic properties of the scaffolds exert significant influence on the direction and inclination of nerve cells, thus explore the prospects of nerve tissue repair [23, 52]. Natural biopolymers among biomaterials are extensively used for nerve tissue-engineering due to their unique advantages over synthetic polymers [174].The properties that provide a natural polymers edge over synthetic polymers include biocompatibility, bioactivity, biomimetic properties, mechanical kinetics and controlled degradation [70, 138, 151].

Keeping in view the fact that cells reside in a 3D niche in their physiological habitat, the 3D electrospinning has been introduced to fabricate nanofibers suitable to provide the cells exactly the same 3D niche as they have in their physiological environment [166]. This 3D natural polymeric scaffold promotes better proliferation, cell adhesion, migration, and differentiation [22]. These scaffolds display the almost similar surface topographies like native tissue, thus influence the cells in a positive way than 2D scaffolds. For the best efficiency of the scaffold function, the natural polymers have been used individually and as composites with other polymers [94]. The various forms of natural polymeric scaffolds so far employed in nerve tissue-engineering include fibrous scaffolds, solid porous scaffolds, polymer gels, acellular scaffolds [43, 105]. Natural polymeric scaffolds so far fabricated for nerve tissue-engineering are alginates [97], chitosan [117], collagen [37], silk fibroin [48], fibrin [10], gelatin [54], gellan gum [150] hyaluronic acid [155]. Further improvements in scaffold architecture have to be performed in order to overcome barriers like cell adhesion, proliferation, proper nutrient supply, cell infiltration and mechanical stability [2, 166]. The natural biomaterials manipulated by functionalization in

nerve tissue-engineering so far include collagen, laminin, silk fibroin, gelatin, gellan gum, fibrin and polysaccharides like chitosan, alginates and hyaluronic acid [49, 141]. Studies show that human neural stem cells adhered to various scaffolds like chitin-alginate have been studied for nerve tissue-engineering [108]. Similarly, mesenchymal stem cells derived acellular matrixes have been used to functionalize composite chitosan/silk scaffold nerve reconstruction [62]. Besides these, ECM functionalized scaffolds/hydrogels containing various biomaterial guides or conduits (e.g., chitosan guides) have also been fabricated with the aim to reconstruct peripheral nerve gap [58]. Moreover, various polymeric scaffolds have also been incorporated with different nanoparticles like poly (3, 4-ethylenedioxythiophene) and graphene oxide [168, 180]. These nanoparticles promote scaffold conductivity that in turn contributes towards physiologically relevant electrical stimulation, which is necessary for guided nerve-regeneration [67, 68, 170]. Furthermore, functionalization of scaffolds with various biomolecules (e.g., proteins) is of key importance in tissue-regeneration and it is helpful in achieving the desired experimental results [141]. Thus, remembering the importance of nerve tissue-regeneration post-injury and the different approaches by which various natural polymeric scaffolds can be tailor-manipulated and functionalized. The current chapter focuses on a brief insight towards nerve-regeneration and importance of these techniques in regeneration followed by detailed, recent progress and possibilities of the polymeric scaffolds in nerve tissue-regeneration.

27.2 Chitosan as Biomaterial

Chitosan (CS) is a polysaccharide derived from the chitin. Chitin is a homo-polysaccharide, found in the exoskeleton of crustaceans, molluscs, and insects, cell walls of green algae, yeasts and mushrooms [36, 137]. CS is obtained from the de-acetylation of the chitin and is composed of monomers D-glucosamine and N-acetyl-glucosamine joined together by a β(1–4) glycosidic bonds [135, 137]. The three functional groups, one amino group and two hydroxyl groups in the structure of CS contribute to its cationic nature and thus promote the affinity for anionic biomolecules [14, 123]. CS is the only natural polymer to possess this characteristic feature [19]. The content of amino groups and subsequent rate of acetylation/de-acetylation of monomers along with solubility, bioactivity, and biodegradability makes chitosan an amazing polymer in various fields, including tissue-engineering [36, 69].

27.2.1 Chitosan Scaffolds in Nerve Tissue-Engineering

CS has been widely explored in nerve tissue-engineering keeping in view its versatility [135, 169]. Guan et al. fabricated a novel composite scaffold with polymers of chitosan/gelatin functionalized with hyaluronic acid and heparin sulfate by freeze-drying technique [63]. Hyaluronic acid and heparin sulfate are glycosaminoglycans, important constituents of ECM. Moreover, the hyaluronic acid has been investigated for playing a considerable role in scaffold designing for tissue-regeneration [81]. It is an anionic biopolymer and has also been investigated for the proliferation of murine neural progenitor cells (NPCs) *in vitro,* together with fibroblast growth factor (FGF-2) [30]. Moreover, hyaluronic acid along with FGF-2 also helps in generation of the neurons from fetal human NPCs at a large scale in the very precise time period [63, 126]. Freeze-drying is the commonly used technique to fabricate porous scaffolds where the freezing rate contributes towards the nucleation and considerable heterogeneity of the scaffold [125]. In the aforementioned study, the three composite scaffolds with varying content of CS/gelatin/hyaluronic acid/heparin sulfate were fabricated. This was followed by analysis of their characteristics in comparison with the pure CS/gelatin scaffold. These scaffolds have been characterized by analysis of their micro-architecture, physiochemical and biological

properties. The pore-size, total porosity and water absorption in composite scaffold showed no significant difference when the CS content was decreased and the content of gelatin with hyaluronic acid and heparin sulfate was increased. However, a significant difference in porosity was observed in comparison with CS/gelatin scaffold. Following the NPCs seeding on these scaffolds and during the subsequent investigation, it was observed that the composite scaffolds of CS/gelatin/hyaluronic acid/heparin sulfate promoted the cell adhesion and cell viability. Moreover, upon proper induction, the neuronal differentiation potentiates considerably compared to CS/gelatin scaffolds [63].

ECM functionalized tissue-engineered nerve grafts are emerging as a primary and potential approach for nerve tissue-regeneration [174]. To overcome the limitations of autografts various artificial conduits or guides of natural polymers have been developed [60]. The nerve gap >15 mm is very difficult to reconstruct [42, 109] hence, various approaches are attempted to overcome this problem [60]. CS has been widely exploited for fabrication of nerve conduits with diverse functionalization. Gonzalez-Perez et al. studied the regeneration power of CS tubes with varying degree of acetylation in the reconstruction of 15 mm sciatic nerve gap in Wistar Hannover rats, compared with silicone tubes and nerve grafts. Compared to the nerve grafts where all rats showed efficient regeneration and reinnervation, about 100%. It was observed 57% in rats with CS guide having (5% acetylation) and with silicone tubes the rats completely failed to regenerate [58]. Considering this generalization, another study fabricated and functionalized the CS conduits with the incorporation of the CS film in the hollow CS conduits. The regeneration outcome was analyzed in the reconstruction of 15 mm long sciatic nerve gap in both healthy and diabetic rats. Results conclude that the regeneration potential of these conduits almost approached the regeneration potential of rats with nerve grafts [116]. Further, exploration of the similar concept by functionalization of CS tubes using collagen enriched with laminin and fibronectin resulting in stabilized and the hydrated scaffolds/constructs. The efficiency of these scaffolds enclosed CS tubes were tested in the reconstruction of 15 mm nerve gap in rats. The study demonstrated that the collagen/fibronectin enriched constructs (stabilized scaffold) have revealed increased myelinated fibers, along with increased SC migration and reinnervation compared to the collagen/laminin constructs (stabilized scaffold) and the corresponding hydrated scaffolds [59]. Following the nerve transection, the SCs at the site of injury, possess the ability of proliferation, differentiation and subsequent migration, thereby acting as guidance cues for axon-regeneration [121]. Keeping this in consideration, transplantation of SCs into nerve conduits has recently been explored and the apparent addition of neurotrophic factors demonstrated axonal and functional recovery of nerves [65, 111, 130]. Further, as one more step towards efficient nerve-regeneration by CS, conduits incorporated with the hydrogels containing engineered SCs have been investigated by Meyer et al. Hollow CS nerve conduits with 5% acetylation and functionalization of the conduit lumen by genetically engineered rat SCs and FGF-2 enriched hydrogel scaffold have been used in this study. Overexpression of glial-derived neurotrophic factors (GDNF) and FGF-2 by the SCs demonstrated that release of FGF-2 promoted the efficiency of regeneration by the conduits. Further, the release of FGF-2 by SCs increased the functional recovery up to 57% in bridging the 15 mm sciatic nerve gap, in comparison to the rats where autografts were implanted. On the other hand, GDNF failed to perform these functions [117].

Nerve cells are electrically active cells, generation of electrical impulse and subsequent transfer of stimuli plays an important role in the functioning of the nervous system [159]. Recent research focuses on exploiting this electrical property of the nervous tissue in the fabrication of the electro-active biomaterial for nerve tissue-regeneration [15, 66]. The electrical, physical and chemical properties of these electro-active scaffolds can be manipulated according to experimental requirements related to the particular application [82]. Polymers such as

polypyrrole, polyaniline, poly(3, 4-ethylenedioxythiophene) (PEDOT) are known for their conductive nature due to efficient electro-optical properties and thus are contributing significantly in various biomedical applications [41, 68, 171]. Due to the hydrophilicity and poor biodegradability of these conductive polymers, they are often utilized in tissue-engineering as blends of natural polymers [64]. Wang et al. fabricated the conductive polymer with PEDOT nanoparticles enriched CS/gelatin scaffold by *in-situ* interfacial polymerization. In this study, the CS/gelatin hydrogel has been used as a scaffold and subsequent enrichment with varied content of PEDOT nanoparticles promoted its electro-optical properties. The analysis of various characteristics of these scaffolds demonstrated the successful enrichment of 50 nm diameter PEDOT nanoparticles on the scaffolds (Fig.27.2). Further, there was an increase in the electrical conductivity, thermal stability besides improved hydrophilicity and mechanical properties compared to pristine CS/gelatin. Consequently, there was a decrease in biodegradation and water absorption when compared to CS/gelatin scaffolds. Biocompatibility analysis indicated that upon cell seeding these scaffolds enhanced the cell adhesion and proliferation of neuron-like rat pheochromocytoma (PC12) cells. The gene and subsequent high protein expression analysis concluded that in these cells the neurite growth was enhanced using these scaffolds and thus suggests that it may prove a significant scaffold in nerve-regeneration [168].

The tailoring of the scaffolds towards their ultimate excellence, various functionalization approaches has been explored towards this goal. Tissue-derived ECM, both neural and non-neural has been explored in the functionalization of neural polymeric scaffolds [28, 89]. These scaffolds have been successful in nerve repair, but counter certain limitations like lack of availability of tissue, poor mechanical properties and uncontrollable degradation [11, 100]. To overcome these limitations, new research approaches explore the possibilities of the cell-derived ECM as a substitution to the tissue-derived ECM [102, 162]. Furthermore, Gu and coworkers [62] functionalized the chitosan nerve conduits by silk fibroin (SF) filaments incorporated with ECM-derived from bone marrow mesenchymal stem cell (BMSC) for nerve repair in rats. BMSCs have been exploited

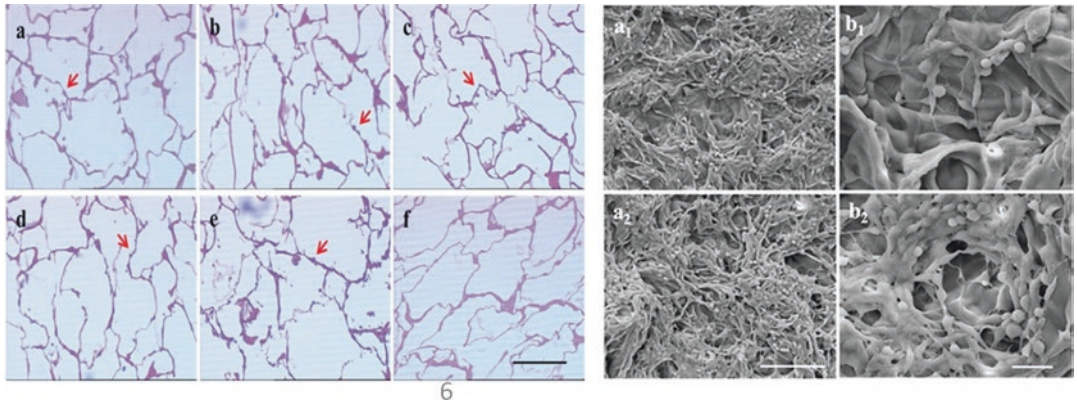

Fig. 27.2 Histomorphological analysis of H&E staining of scaffold constructs after 3 days of culture; (**a**) CS/Gelatin; (**b**) 0.5 PEDOT/CS/Gelatin; (**c**) 1PEDOT/CS/Gelatin; (**d**) 2PEDOT/CS/Gelatin; (**e**) 4PEDOT/CS/Gelatin; (**f**) CS/Gelatin devoid of cells: scale bar = 200 μm and scanning electron microscopy images of scaffold constructs of CS/Gelatin without (a_1 &b_1) and with PEDOT(2PEDOT/CS/Gelatin) and cells (a_2 & b_2); scale bar of (a_1, a_2) & (b_1, b_2) =200 μm and 50 μm, respectively, demonstrating successful enrichment of 50 nm diameter PEDOT nanoparticles on the scaffolds. (Reprinted with copyright permission from Royal Society of Chemistry [168])

extensively in tissue-engineering due to their ability to differentiate into SC-like cells, both *in vitro* and *in vivo* [33, 107]. Scaffolds both CS conduit and SF have been prepared and subsequently cultured with BMSCs in this study. This was followed by the de-cellularization of the BMSCs thus, results in the production of ECM functionalized CS-SF scaffold. Results declared that this scaffold potentiates the regenerative process of implantation in rats to bridge the 10 mm long sciatic nerve lesion compared to pristine CS-SF scaffold [62]. Adding more to this, Xue et al. utilized the dog BMSCs-derived ECM to functionalize the CS-SF scaffold. The de-cellularization of the BMSCs onto these scaffolds was used to reconstruct the 60 mm sciatic gap in dogs. Histological analysis of this scaffold after implantation revealed the promotion of regeneration and reinnervation processes. Further, it was observed that the regeneration output was similar to that of autografts [174].

27.3 Collagen as Biomaterial

Collagen the main structural protein in vertebrates has been extensively investigated for tissue-engineering. Collagen is composed of three polypeptide strands helically wound about each other, forming the triple-stranded α helical structure [24, 176]. Following synthesis from the endoplasmic reticulum and subsequent delivery to ECM, collagen fibrils assemble in the form of a scaffold-like structure by cross-linking with each other for this enzyme lysyl oxidase plays a significant role [83]. Collagens besides establishing structural unity by making ECM and maintaining functional aspects of the connective tissues present there are also responsible for proper cell adhesion, proliferation, differentiation, migration and cell viability [20, 176]. Collagens being the most abundant constituent in ECM have been widely explored for general tissue-engineering including nerve tissue-engineering.

27.3.1 Collagen as a Scaffold for Nerve Tissue-Engineering

The investigations for using collagen as a biomaterial scaffold for nerve tissue-engineering are increasing in recent years [48]. The properties of the collagen especially its abundance in ECM has made it the most explored polymer in tissue-engineering. Besides the functionalization of scaffolds with various biomolecules, the functionalization with NPCs has also gained importance [131]. The NPCs as cells for functionalization are efficient in regenerating neural tissues as they have the capacity of self-renewal and ability to differentiate into various glial cells [134]. In this context, the regeneration power of the hNPC-derived astrocytes (hNP-AC) adhered on collagen scaffolds were fabricated by a directional freezing process, followed by their interactive studies with the migrating SCs and fibroblasts. Both 2D and 3D cultures used in this study concluded that human neural progenitor cells hNPCs possess strong and effective axon-regeneration power in dorsal root ganglia. Following cell seeding, a homogenous distribution of the hNP-AC was seen onto the 3D collagen scaffolds (Fig. 27.3) [52]. The axon growth was enhanced by cell seeding when compared to non-seeded scaffolds. Further, interactive studies of SCs and the fibroblasts with the hNP-AC revealed strong intermingling of these cells. Therefore, demonstrates the effective interaction between the hNP-AC, SC, and fibroblasts. These results are contradictory with general properties of SCs/astrocytes and fibroblasts/astrocytes association where they are found in distinct localization [104, 144]. Thus, investigation demonstrated that the 3D collagen scaffold surface morphology substantially excluded the property of CNS and PNS, cells from being distinctive in location and thus this property can be of great significance for implantation and for potentiating the axon-regeneration post-nerve injuries (Fig. 27.4) [52].

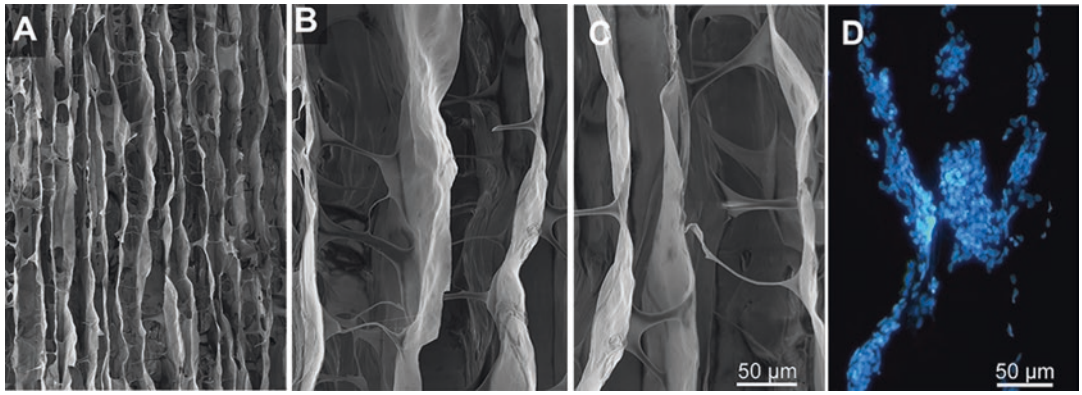

Fig. 27.3 SEM micrographs A (scale bar 200 µm), B and C (scale bar 50 µm) of the 3D collagen scaffold representing the micro-architecture of scaffold. Image D represents the DAPI labeled hNP-AC cells. (Reprinted with permission from Elsevier [52])

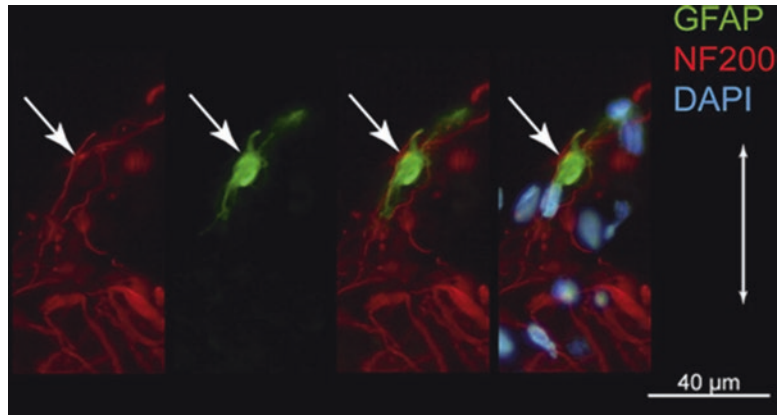

Fig. 27.4 Representation of GFAP positive (green) hNP-AC associated with regenerating axon bundles NF200 positive (red). Double headed arrow represent micro-channel orientation reprinted with copyrights permission from Elsevier [52]

Similarly, a 3D scaffold of collagen I for mimicking the nervous tissue was developed and functionalized with the rat NPCs [53]. For the proliferation of these cells, the induction with epidermal growth factor and basic FGF-2 support the neuron growth and promotes the differentiation of the cells into neurons and astrocytes [127]. Recently, the composite scaffold was developed by exploiting the collagen, hyaluronic acid and alginates. The scaffold was prepared by the self-assembly fabrication process with methacrylic anhydride functionalization succeeded by photo-crosslinking and grafting with GRGDSP/Ln5-P4. The corresponding characteristics of the scaffolds with varied content revealed that (collagen/methacrylic anhydride, hyaluronic acid/methacrylic anhydride and alginates/methacrylic anhydride) in the ratio (1:2:1) proved to be optimum concentration. Upon seeding with induced pluripotent stem cells this scaffold stimulated the differentiation of these cells into neurons, thus demonstrated that it can be used as a potential differentiation inducing biomaterial for regeneration [94]. Besides, the uses of collagens as a main polymer in the scaffolds its application has been explored in the development of the 3D microfluidic system for neuronal differentiation. For instance, the 3D collagen hydrogel was fabricated to immobilize the hNSCs and subsequently used to occupy the central channels in the microfluidic device. Further, the aim of this study was to evaluate the differentiation signals coming from the human mesenchymal stem cells hMSCs overexpressing the GDNF that inhabited the channels surrounding the microfluidic device containing hNSCs. The resultant neuronal cells

differentiated under the influence of the hMSCs, exhibited neuron-like features. The *in vivo* results of this study revealed that the 3D microfluidic device can be used as an efficient material for investigation of signals from transplanting stem cells to stimulate endogenous neuronal behavior of the hNSCs [177]. Moreover, collagen I was also utilized for the functionalization of the polycaprolactone (PCL) nano-fibrous scaffold fabricated by electrospinning technique [172]. PCL being the polymer with good mechanical properties [76], and its efficiency to hold neural cells *in vitro* and *in vivo* are well known [165]. MSCs derived from the Wharton's jelly were seeded and then subsequently differentiation by using retinoic acid and sonic hedgehog promoted them into motor neuron-like cells [12]. Furthermore, the incorporation of the collagen as a graft into scaffolds revealed the enhanced differentiation potential. These studies reveal the varied dimensions and prospects of the collagen to be used in nerve tissue-regeneration.

27.4 Hyaluronic Acid as Biomaterial

Hyaluronic acid, a heteropolysaccharide is a non-sulfated glycosaminoglycan (GAG) which is composed of repeating disaccharide units of [acidic sugar and amino sugar]. The acidic sugar in the hyaluronic is D-glucuronic acid and amino sugar is N-acetyl-D-glucosamine [84, 142]. Besides, the role of the hyaluronic acid in immobilization of the various drugs, antibodies, growth factors for their controlled release, it has been widely explored for varied applications in wound healing, intra-dermal implants and in nerve tissue-engineering [47, 72].

27.4.1 Hyaluronic Acid Scaffolds in Nerve Tissue-Engineering

Hong et al. demonstrated the hyaluronic acid -catechol conjugate can be used in the fabrication of the hydrogel scaffolds [74]. Catechol, present in the proteins of the marine mussels *Mytilus edulis* possesses both adhesive and cohesive properties [163]. The adhesive and cohesive properties play important role in functionalization of the scaffolds [27]. Hong and co-workers developed a novel polymeric conjugate by dopamine coupling with the COOH group of the hyaluronic acid at pH 6 that explored the properties of both hyaluronic acid and catechol. Following characterization, the conjugate showed efficient adhesive and the cohesive properties under acidic (pH less than 2) and alkaline conditions (pH 8–9) respectively. Further, in this study, cross-linking between the catechol moieties in the conjugate solution was induced upon addition of oxidizing agent (i.e., sodium periodate) under alkaline conditions thus, results in the formation of the hydrogel. This lyophilized hydrogel was then used as a scaffold for culturing of the hNSCs. Various synthetic polymers functionalized with this conjugate revealed that hyaluronic acid-catechol conjugate promotes the cell adhesion and differentiation, thus can be considered as a biopolymer for culturing hNSCs for used in nerve tissue-regeneration [74]. The 3D layered hydrogels can also be fabricated by density gradient multilayer polymerization which involves modification of the cell suspension containing polymer by small molecules that act as density influencers [86]. Therefore, in another study, Zhang et al. explored the same approach for their study [179]. The methacrylate functionalized hyaluronic acid was used followed by varied exposures of ultraviolet A to produce hydrogels with different firmness. Based on this, the hydrogels with pore size 10 μm and firmness 100 Pa were used further in this study. The ultimate aim of this study was to investigate the movement of the NPCs towards various glial cells like astrocytes/neurons. Further, investigation of these movements in patients with Rett syndrome (i.e., genetic X-linked syndrome with the mutation in the methyl-CpG binding protein-2 gene) was carried [6]. This gene governs the neuronal development and mutation in this can halt the development process and thus results in neuro-developmental syndrome [32]. So, a comparative investigation based on the migration of the NPCs derived from the induced

pluripotent stem cells (iPSCs) and mutant iPSCs demonstrated that upon induction by either astrocytes or neurons the mutant iPSCs-derived NPCs reveal halted migration. During the harvesting mature neurons confirmed flawed synaptogenesis and neurite outgrowth compared to wild-type NPCs which yield electro-active neurons [179]. This excellent work demonstrated the application of hydrogel scaffolds in monitoring defects associated with various disorders besides their role in regeneration. Therefore, exploring the various dimensions of the polymeric scaffolds to be used in regenerative medicine.

Another step towards finding solutions to various challenges to overcome barriers faced in nerve tissue-regeneration. The development of hyaluronic acid/laminin hydrogel was prepared by using thiol group functionalized hyaluronic acid followed by its cross-linking to poly (ethylene glycol) divinyl sulfone with laminin [1]. This was followed by fabrication using NPCs obtained from the medial and lateral ganglionic eminences of mice. The broad aim of the study was to evaluate the hyaluronic acid/laminin hydrogel scaffold for the retention of the transplant and migratory response to SDF-1α *in vivo* using mice as animal models. The SDF-1α also called as CXCL12 is the strong chemokine involved in directing the cells during the developmental process. The interaction of the SDF-1α with its receptor CXCR4 guides migration of the germ cells besides its role in the immune cell development process [44]. Moreover, the signaling cascade triggered by the binding of SDF-1α with its receptor CXCR4 is necessary for the preservation of BMSC and NSC niches at post-development stages [153, 160]. It has been reported that SDF-1α involved in mobilizing the marrow-derived stem cells and NPCs towards injury sites [77]. The transplantation of NPCs within HA/laminin hydrogel potentiates the retention of cells significantly in comparison to bolus transplantation of NPCs on 1st and 3rd days. Moreover, upon exogenous introduction of the SDF-1α just after the NPCs transplantation, the scaffold promotes the NPCs migration significantly towards the SDF-1α [1].

Another study indicated the nerve-regeneration potential of the human periodontal ligament stem cells (PDLSCs) and gingival MSCs after the fabrication of 3D scaffolds [8] PDLSCs being present in the oral cavity and in the tissue wastes of dental clinics, various researchers have confirmed their capacities for multi-lineage differentiation [119, 120]. The hyaluronic acid/alginate hydrogel of varied alginate/hyaluronic acid contents were investigated and the scaffold with alginate: hyaluronic acid content in the ratio of 1:1 showed lowest elastic modulus compared to other hydrogels including the alginate hydrogel alone. The proliferation study of the cells revealed that the high proliferation rate of the gingival MSCs corresponds to the hydrogels with a decrease in elasticity. However, no significant difference in the proliferation of the PDLSCs and hBMSCs was revealed with varying elasticity. This study concludes that alginate/hyaluronic acid scaffolds can prove a potential efficiency to be used in the nerve-regeneration [8]. To promote excellent cell adhesion, proliferation and the differentiation of the neural tissue scaffolds the efficient porosity of the scaffold is mandatory for a bio-mimicking [7]. Recently, as a step to improvise the aforementioned property of the engineered scaffolds, the study evaluated the use of the potassium di-hydrogen phosphate commonly called as urea crystals to induce pores to the hyaluronic acid hydrogels. These urea-templated hydrogels were investigated for bioactivity upon seeding with the NPCs and SCs. The results reveal that NPCs has shown minimal differentiation in templated scaffolds thus preserving their undifferentiated status compared to the non-templated control hydrogels. On the other hand, the SCs cultured on the template-scaffold showed differentiation (though at lower rates) compared to controls [155]. This work suggests that efficient and judicious application of this approach with various biomolecules acting as a sacrificial template can be explored to create scaffolds architecturally similar to neural ECM for better regeneration outcomes.

27.5 Silk Fibroin as the Biomaterial

Silk a natural macromolecular polymer is synthesized by various *Lepidopteran larvae* in specialized glands and the following secretion into the lumen of the epithelial cells, it is spun into the fibers. The SF and the sericin are the main proteins present in the silk with SF occupying core position and the sericin marks out its periphery [5, 143, 161]. Mostly the SF-derived from the mulberry silkworm *Bombyx mori* has been commonly used so far in tissue-engineering [87, 145].

27.5.1 Silk Fibroin as a Scaffold for Nerve Tissue-Engineering

However, SF from the non-mulberry silkworms such as *Antheraea mylitta* has also been explored for biomedical and bio-engineering applications. In this context, Subia et al. used the freeze-drying approach of fabrication of scaffolds to evaluate the potential of SF derived from both the sources *Bombyx mori* (mulberry) and *Antheraea mylitta* (non- mulberry) for acting as a scaffold for hNPCs. Following fabrication, the characterization of the scaffolds revealed that the pore size and porosity of the scaffold do not show any significant difference. Hence, both are efficient for cell seeding. Moreover, hNPCs following seeding were investigated for 14 days, comparatively on both the scaffolds and results demonstrate that both have good cell viability and ability to potentiate the proliferation with a substantial increase in the hNPCs marker nestin. Further, there was no significant difference between the results shown by the two scaffolds except for scaffold prepared from the non-mulberry source show a minimal increase in the cell proliferation [152]. Adding more, Xu and coworkers developed the composite scaffold of SF and collagen for sciatic nerve-regeneration in the rats [173]. In this case, the SF and collagen hydrogel was prepared separately following the method elsewhere [140, 146]. Then for the composite scaffold preparation the SF: collagen solution in the ratio of 4:2 was injected into the casting mold and via lyophilization, de-moulding was achieved. The functionalization of the scaffold was achieved by the SCs isolated from the neonatal rat sciatic nerve and adipose-derived stem cells (ADSCs) obtained from the inguinal region of adipose tissue. After characterization, these functionalized scaffolds were transplanted in the sciatic nerve of rats after a surgical procedure. The electrophysiological examination after implantation revealed no significant variations in compound muscle action potential amplitudes and motor nerve conduction velocity between the rats transplanted with the tissue-engineered scaffold and those bearing autografts. However, these results were significantly lower in rats transplanted with the pure scaffold. Moreover, scaffolds were successfully applied to bridge the 1 cm gap in sciatic nerve of rats (Fig. 27.5) [173].

The SF conduits and simultaneous functionalization of the lumen in the conduit by SF fibers were achieved by the process of the electrospinning by Xue and colleagues [175]. The aim was to test these conduits for the reconstruction of the 10 mm gap in the sciatic nerve in dogs. Post-surgery, the dogs were investigated for 12 months for behavioral and functional recovery and the results demonstrated that SF-based scaffolds revealed almost the same results as revealed by dogs transplanted with autografts. Thus, the study declared that SF-based scaffolds can efficiently be used as a possible alternative for nerve-regeneration. Dual functionalization by the brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) of SF scaffolds fabricated by electrospinning technique was achieved by Liu et al. [103]. The BDNF is a neurotrophic factor and is responsible for promoting the nerve-regeneration process [3]. On the other hand, VEGF is a strong growth factor involved in the process of vascular permeability and angiogenesis [3]. Moreover, SCs were used in this study for the biocompatibility and bioactivity analysis of scaffolds and factors respectively. Following the implantation in the mouse model, the results revealed the increased revascularization and nerve-regeneration compared to the pristine SF scaffold (i.e., without factors) at 4th and 8th weeks post-transplantation (Fig. 27.6) [103].

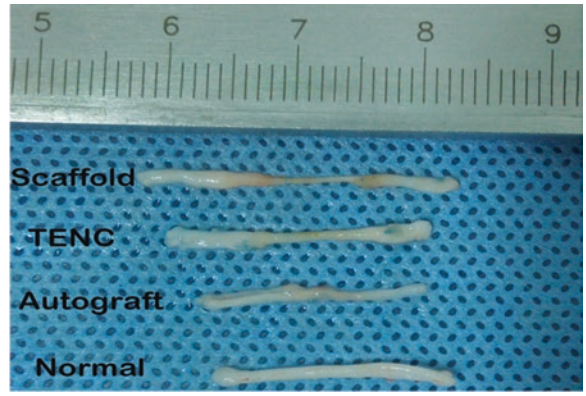

Fig. 27.5 Representation of regenerated nerves after implantation with plain SF/Collagen scaffold, tissue-engineered nerve conduit (TENC), autograft and, control rats while investigating the reconstruction of 1 cm nerve gap in sciatic nerve. [173]

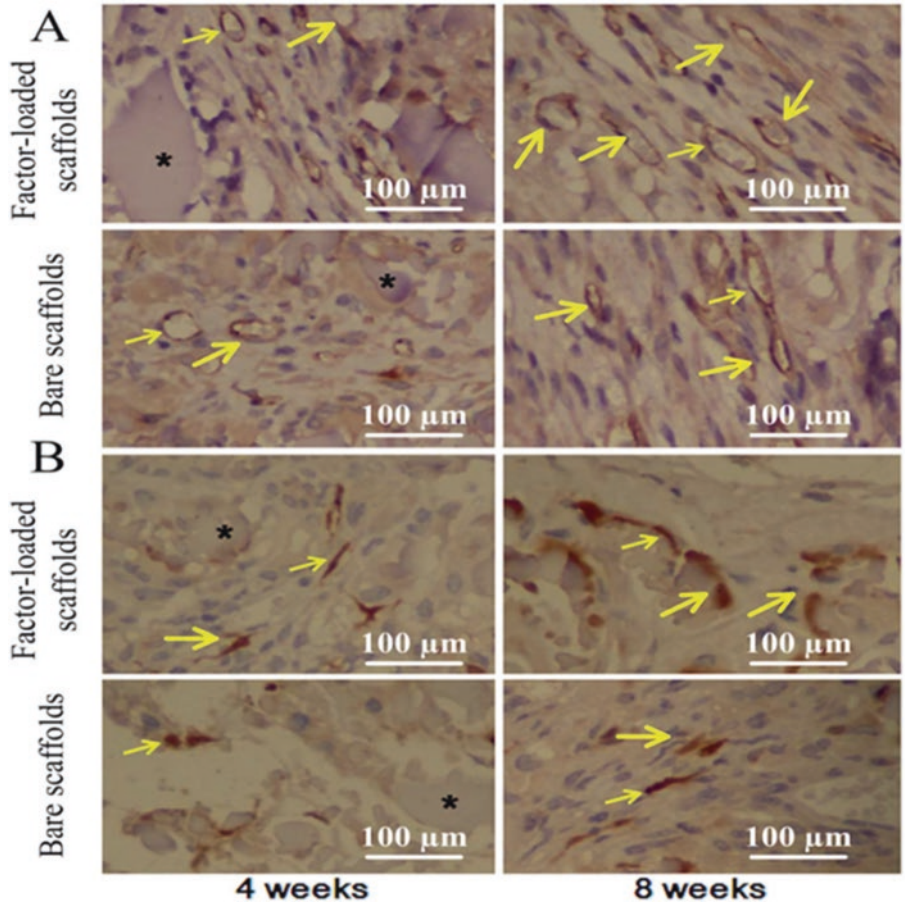

Fig. 27.6 Immunohistochemical staining for vessel evaluation at the panel (**a**) and innervation in SF scaffolds with and without dual factors at 4 and 8 weeks post-implantation at the panel (**b**). The asterisk sign denotes scaffold fragments and yellow arrows the vessels and neuronal lineages with copyright permission from Royal Society of Chemistry [103]

Recently, another step towards overcoming the problems faced in the regeneration process after an event of injury, Zhao and co-workers fabricated the SF scaffold by electrospinning. Furthermore, the simultaneous incorporation of graphene-oxide was achieved by sonication thus, resulting in the composite graphene-oxide enriched SF scaffolds [180]. Graphene is a carbon-based 2D material with a honeycomb-like lattice configuration, having a single layer of sp_2 hybridized carbon atoms [71]. Besides, exploring various avenues in different fields such as targeted delivery, biosensing, detection and use in various electro-optical and storage devices [181]. The pitfalls like high hydrophobicity, lack of biocompatibility and the low solubility limit its applications in certain fields, including tissue-engineering [178]. Keeping in view, the electro-active property of the graphene and that of nervous tissue (i.e., being electrically-active) Zhao and colleagues explored the incorporation of the graphene in a polymeric scaffold for nerve tissue regeneration. Upon investigating the various characteristics of scaffolds with varied content of graphene, the scaffold with 10% was declared efficient for further use. Moreover, upon seeding of SCs onto this scaffold the cell adhesion, survival and proliferation were carried efficiently. This study thus provides the inspiration to manipulate the scaffolds with electro-active materials for efficient regeneration results [180]. Besides the exploration of various natural polymers in nerve tissue regeneration, the blends of natural/synthetic polymers have also been widely applied in regenerative processes including nerve regeneration [145].

In this context, the poly (lactic acid) (PLA)/SF composite scaffold was fabricated and incorporation of the nerve growth factor (NGF) was achieved by co-axial electrospinning. Moreover, the plasma treatment [178] after the fabrication was given and the scaffold was further studied for the sustained release of the NGF. The differentiation of the PC12 cells was also investigated onto the scaffolds and the results confirmed the ability of the plasma-treated scaffolds as a suitable substrate for regeneration compared to the pure scaffold (i.e., devoid of plasma treatment) [157]. Similarly, the composite scaffold of SF derived from the *Antheraea pernyi* [178] and poly(L-lactic acid-co-caprolactone) was fabricated by electrospinning. Following the various evaluations, this composite scaffold was declared significantly efficient to support cell survival and proliferation compared to poly(L-lactic acid-co-caprolactone) co-polymer alone upon seeding with SCs [167]. Adding more, the investigations of various characterizations and biocompatibility/bioactivity testing of the laminin functionalized SF/poly(ethylene oxide) scaffolds after seeding of SCs reveal their application in nerve regeneration process [132].

27.6 Gelatin as Biomaterial

Gelatin is a natural polymer and is obtained by partial hydrolysis from the collagen. It is a soluble fibrous protein and is a constituent of the bones, cartilages, and skins [75]. The abundant sources of gelatin are pig skin, bovine hides, cattle and fish bones with the approximate concentration of 46%, 29.4%, 23.1% and 1.5%, respectively [56, 85]. Besides the biocompatibility and biodegradability, the gelatin is easily available and less expensive commercially, therefore, it has been widely used in bio-engineering field [34, 92].

27.6.1 Gelatin as a Scaffold for Nerve Tissue-Engineering

In this regard, gelatin extracted from genipin was successfully electrospun into nanofibers using electrospinning technique. Further, this gelatin was enriched with ECM derived from the de-cellularized brain of rat [13]. The broad aim of their study was to evaluate this ECM enriched scaffold in nerve tissue-engineering. Following the fabrication and succeeded by the cross-linking with gelatin the biocompatibility and bioactivity analysis of these scaffolds was performed by seeding with rat MSCs. The results reveal the cytocompatible property of these scaffolds for MSCs and marrow mononuclear cells. The hematoxylin and eosin staining

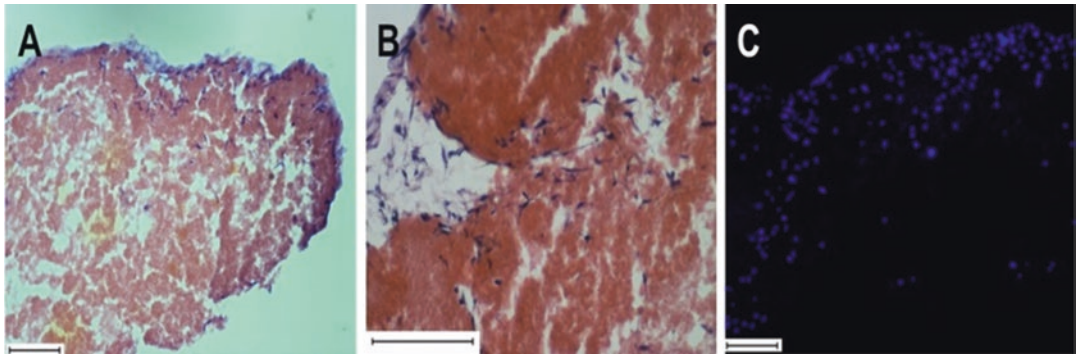

Fig. 27.7 (**a**) Hematoxylin, (**b**) Eosin and (**c**) DAPI staining of decellularized brain-ECM with rat MSCs seeded, scale bar = 25 μm with copyright permission from Elsevier [103]

revealed the expected fibroblast-like extended morphology and DAPI staining reveals the proliferation of both cell types on the ECM enriched mats (Fig. 27.7). This *in vitro* study shows the potential modality to be explored in near future for *in vivo* studies so that it can be regarded as a step in the context of tissue-engineering towards CNS regeneration [13]. Gnavi et al. proposed another step towards the nerve-regeneration process. In this case, gelatin hydrogel scaffolds were encapsulated with VEGF to screen the ability for controlling the release of the cargo and role of enclosed VEGF in nerve tissue-engineering [54]. The agar/gelatin composite in the ratio 20:80 wt.% were prepared and followed by the cross-linking with the genipin [158]. Following this, the incorporation of the VEGF-A165 was initiated [129]. The release of the VEGF from the hydrogel was investigated by ELISA immunoassay and it was confirmed that it is successfully released from the hydrogels. For bioactivity analysis of the VEGF post-release studies, the human umbilical vein endothelial cells (HUVECs) were used. The screening ability of the VEGF to phosphorylate its downstream effectors like VEGFR-2, Erk1/2 and Akt in its signaling cascade successfully revealed its bio-activity. It was also demonstrated that VEGF-A165 released from hydrogel maintains its angiogenic effect upon investigation on HUVECs [21] and potentiates the axon outgrowth as evaluated on the DRG explants derived from the adult female Wistar rats. Further, investigation of the scaffold enriched with VEGF-A165 was carried by seeding of DRG explants on the hydrogel and results demonstrate that neurite growth was increased. This proves that gelatin-based hydrogels are an efficient source for encapsulation of bioactive molecules for application in regenerative medicine [54].

The encapsulation of various factors in polymeric scaffolds has been implicated further in bio-engineering. For example, the NGF encapsulated with alginate microspheres and subsequently, their integration in nano-fibrous gelatin scaffolds was achieved [110]. This approach utilizes the properties of both the alginates and gelatin in tissue-engineering process. These nano-fibrous scaffolds were prepared by phase separation technique [112], under the influence of paraffin which acts as a porogen. Following the characterization, these scaffolds were evaluated for the regeneration process by seeding of PC12 cells derived from the rat adrenal. The results demonstrate that microsphere loaded scaffold revealed the controlled release of the bioactive NGF, as a result, the neurite growth was observed in PC12 cells. Therefore, suggesting that this scaffold may have potential application in exploring various studies towards nerve-regeneration in CNS [29].

27.7 Alginates as Biomaterial

Alginate is a natural anionic polymer, obtained from *Phaeophyceae* (brown algae), including *Laminaria hyperborea, Laminaria digitata, Laminaria japonica, Ascophyllum nodosum* and *Macrocystis Pyrifera* [156]. Moreover, the alginates are also synthesized by some bacterial species, including *Azotobacter* and *Pseudomonas.* Alginates synthesized by these bacterial species furnish the well-defined chemical structure and physical properties compared to alginates derived from brown algae. They have been widely studied due to their potential applications in the bio-engineering field.

27.7.1 Alginates as a Scaffold for Nerve Tissue-Engineering

The mild gelation properties of alginates in the presence of divalent cations besides the biocompatibility and low toxicity have made it a suitable candidate to explore its applications in various forms of scaffolds for use in tissue-engineering [95]. These gelling properties have explored many avenues and thus have paved various ways towards the utilization of this property in the fabrication of scaffolds. Li et al. utilized this gelling property of alginates to encapsulate NSCs [97]. In this case, sodium alginate and calcium chloride in varying proportions were prepared and left for gelling to trigger the formation of the beads. After encapsulation of the cells in calcium-alginate, the various parameters like gelling conditions, cell distribution and proliferation were analyzed for the formation and dissociation of the beads. Moreover, the beads with the diameter 2 μm were prepared with 1.5% sodium alginate and 3.5% calcium alginate solution with gelling time for 10 minutes was found efficient for culture. The harvest rate of over 88.5% and the population of the cells encapsulated almost increased two fold during the process. Results demonstrated that these beads can be used as a potential avenue for cell expansion as a 3D scaffold for regeneration [97]. Similarly, alginate micro-beads enriched with the embryonic stem cells were investigated and upon induction with retinoic acid, the differentiation of the stem cells into neural lineage potentiates [108]. Lu et al. fabricated fibrous scaffold by the interfacial electrostatic interaction of sodium alginate and chitin and simultaneously human pluripotent stem cells were immobilized on them. Following the induction, with the appropriate neural markers noggin/retinoic acid the cells expressed neural progenitor markers thus results in the formation of the mature neurons. Upon implantation in the severe combined immunodeficiency SCID mice, these neurons act efficiently without tumor formation, thus opening new ways of manipulating stem cells for nerve tissue-regeneration [108]. Moving further ahead, the alginates have been applied to immobilize the hNSCs and for investigation of the growth, expansion and differentiation of these cells, the 3D cellular microarray platform was developed. The investigations revealed the expansion of hNPCs and cell survival (though slowly) than conventional 2D scaffolds. Further, differentiation into glial cells was revealed, albeit decrease in neural progenitor markers. Moreover, this approach was utilized to screen the toxicity effects of various molecules on hNSCs [114].

27.8 Other Polymers in Nerve Tissue-Engineering

There are several other polymers that have been exploited for tissue-engineering, specifically nerve tissue-engineering such as (e.g., gellan gum and fibrin) besides the aforementioned natural polymers. Gellan gum is an anionic polysaccharide produced by bacteria *Sphingomonas elodea* and is approved by the Food and Drug Administration and European Medicines Agency [51]. Gellan molecule upon de-acetylation yields a tetrasaccharide of repeating units of β-D-glucose, β-D-glucuronic acid and α-L-rhamnose in the ratio of 2:1:1 [118]. It has been also explored for engineering scaffolds for tissue-engineering in recent years [50]. The pitfalls like weak mechanical properties of the traditional

methods [96]. The ionotropic cross-linking and chemical cross-linking for the synthesis of the gellan gum-based hydrogel scaffolds gave existence to an alternative methodology [35, 147]. The cross-linking with biological amines spermidine (SPD) and spermine (SPM) was achieved by the Koivisto and colleagues [93]. These amines are cations in nature and are known to interact with the anionic polymers, for example, gellan gum [106]. Moreover, they serve as cross-linking agents besides their roles as a scavenger in the protection of DNA and in cell proliferation [90]. The reports indicated that at 37 °C these bio-amines act as cross-linking agents after the synthesis of the hydrogels. Further, Koivisto and coworkers achieved the simultaneous functionalization of hydrogels with the laminin. This was succeeded by both encapsulations as well as seeding of the hNPCs and human embryonic stem cells and iPSCs on the hydrogels. After seeding both the SPD and SPM, the cross-linked hydrogels were investigated. Results declare that both hydrogels promotes the cell migration in both the cases and demonstrate that gellan gum hydrogel with 3% SPD concentration stand out from the rest of the hydrogels in the study and thus can prove as a potential candidate for application in regenerative studies [93]. Further, the peptide (GRGDS) functionalized hydrogels of gellan gum has been fabricated for use in tissue-engineering [148]. Gomes and colleagues explored this gellan gum-based hydrogel in the investigation of lumbar spinal cord injury in rats [55]. After synthesis, the bioactivity of gels was tested by encapsulation of the olfactory ensheathing cells derived from neonatal rats and hADSCs [46, 149]. Upon evaluating the *in vitro* studies reveal that co-culturing these cells in hydrogel scaffolds enhanced the neurite growth efficiently. The *in vivo* experiments using the rat as an animal model demonstrate that upon transplantation of gellan gum-GRGDS hydrogel following injury, there was a significant motor recovery in comparison to hydrogel devoid of cells. These results suggest promising gains achieved by gellan gum-GRGDS encapsulated hydrogels [55].

A step towards eliminating the challenges faced by CNS regeneration post-injury, various biomaterial scaffolds are being exploited and evaluated for overcoming these challenges. In this context, Arulmoli et al. developed a multi-polymeric scaffold for the tissue-engineering purpose [10]. The composite scaffold of fibrin, hyaluronic acid, laminin has been derived from salmon and explored for both neural and vascular tissue-engineering. Fibrin an ECM protein during coagulation cascade is involved in the formation of clots. Besides being biocompatible and non-toxic the fibrin possesses RGD sequence in addition to various adhesion sites. The cleavage of the fibrinogen by thrombin generates fibrin monomers that can be explored for the scaffold designing [99]. The degradation rate of fibrin scaffolds during *in vivo* transplantation is only for a few days so mostly fibrin composite scaffolds are preferred. After the scaffold preparation and characterization, the results revealed that hydrogel scaffold prepared from hyaluronic acid efficiently promoted the axon growth than fibrin alone. Further, the multi-polymeric scaffold support human NPCs viability, proliferation, and differentiation, thus can be used in nerve tissue-regeneration. Moreover, the vascularization potential of the scaffolds was investigated by human cord blood-derived endothelial cells cultured alone with scaffolds and in combination with NPCs, the results concluded that in co-cultures the vascularization improves significantly. It was also revealed that salmon-derived fibrin potentiates the proliferation of the NPCs than the bovine and human fibrin [10].

27.9 What Is Coming Next?

Tissue-engineering has explored the various ways of fabrication and functionalization of wide-range of scaffolds for nerve tissue-regeneration. However, besides the extensive research carried out in nerve tissue-engineering until now no approved cell-based polymeric tissue implant for application in nerve regeneration are available. Keeping in consideration the impaired sensory and motor ability due to various nerve injuries and the

treatment challenges related to various neurodegenerative disorders, the clinical translation of functionalized polymeric scaffolds is mandatory. In this context, all researchers should further explore their studies towards this goal. Moreover, the extensive research is currently going on towards the realization of this goal and hopefully, we may be able to counter various challenges in nerve tissue-regeneration in the near future.

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