CHAPTER 10

Hybrid and Composite Biomaterials in Tissue Engineering

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Summary

Biomaterials play a critical role in the success of tissue engineering approaches, as they guide the shape and structure of developing tissues, provide mechanical stability, and present opportunities to deliver inductive molecules to transplanted or migrating cells. Therefore, the selection of the appropriate biomaterial can have a profound impact on the quality of newly formed tissue. A major challenge facing the field of tissue engineering is the development or identification of materials capable of promoting the desired cellular and tissue behavior. Given that few biomaterials possess all the necessary characteristics to perform ideally, engineers and clinicians alike have pursued the development of hybrid or composite biomaterials to synergize the beneficial properties of multiple materials into a superior matrix. The combination of natural and synthetic polymers with various other materials has demonstrated the ability to enhance cellular interaction, encourage integration into host tissue, and provide tunable material properties and degradation kinetics. In the current review, we describe the selection and utilization of numerous hybrid and composite materials to promote the formation of bone, vascular, and neural tissues. The continued development and implementation of hybrid biomaterials will lead to further successes in tissue engineering and regenerative medicine.

KEYWORDS: Composites, Biodegradable polymers, Bioceramics, Inductive factors

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Topics in Multifunctional Biomaterials and Devices, Ed. N Ashammakhi © 2008.

1. INTRODUCTION

Tissue engineered therapies are necessary due to the lack of clinical treatments capable of restoring full functionality once a defect has occurred. One strategy to promote the regeneration of healthy tissue involves the implantation of material-cell hybrid constructs into lesions incapable of self-repair. Although a few tissue engineered products have managed to translate to practicing medicine, most have stalled in the laboratory as a result of unsuitable mechanical, biological, and fabrication properties. Many researchers have tried to resolve these challenges by seeking out new biomaterials, cell sources, or inductive factors to increase appropriate regrowth for the replacement of diseased or damaged tissues. One particular strategy combines previously characterized biomaterials to create composites possessing beneficial attributes not present in its constituent components.

The term '*composite*' is taken in its common form as meaning a structure consisting of two or more distinct parts. This definition is not applied to the molecular level and thus homogenous scaffolds comprised only of co-polymers are not considered within this review. This review presents examples of tissue engineered composites applicable to bone, vascular and neural systems

2. COMPOSITES IN BONE TISSUE ENGINEERING

Although autograft bone remains the current gold standard for treatment of nonunion bone defects and critical sized fractures, it is challenged by a limited supply of viable donor tissue, the need for additional surgeries, increased risk of infection, and donor site morbidity (1). Allograft bone is an alternative to autografts, but these transplants suffer from concerns related to limited donor supply, disease transmission and inadequate physiologic and biomechanical responses (2, 3). Metals and bioceramics have yielded limited successes yet substantial mismatch between their properties and bone tissue persist, thereby punctuating the need for tissue engineered products (4-9). Additionally, inductive proteins cannot be embedded within a metal, necessitating a coating to allow controlled factor release (10). However, metals commonly induce stress shielding and will eventually experience wear debris, ultimately leading to implant failure (11). The ideal tissue engineered construct is a porous interconnected structure that allows cells to migrate and function within its confines (osteoconductive), provides factors that stimulate the proliferation and differentiation of progenitor or osteogenic cells (osteoinductive),

and is capable of assimilating into the surrounding tissue (osseointegrative), eliminating the potential for infection (12-14). Thus, the superposition of two or more materials in order to completely achieve these characteristics is a logical strategy. In effect, the creation of composites is a biomimetic approach, as bone can be viewed as a composite of collagen, the principal organic component; hydroxyapatite, the inorganic mineral component; water; and small amounts of other organic phases (15). Not surprisingly, improvement in regeneration has been observed in composite constructs mimicking the composition and structure of bone.

Increasing interest has been shown in ceramic-polymer composites as potential fillers of bone defects (16-19). Two of the most commonly used calcium phosphate ceramics, tricalcium phosphate and hydroxyapatite, have demonstrated adequate biocompatibility and suitable osteoconduction and osseointegration (20). Bioceramic glasses such as 45S5 Bioglass® have also exhibited the capacity to induce bone-bonding, and even vascularization (21, 22). However, these ceramics are considered too stiff and brittle to be used alone (23). The addition of a ceramic to a polymer scaffold has several advantages including combining the osteoconductivity and bone-bonding potential of the inorganic phase with the porosity and interconnectivity of the three-dimensional construct. The most prominent natural polymer used to fabricate matrices in composites is collagen type I, probably due to its prevalence in bone's extracellular matrix and its ability to promote mineral deposition and provide binding sites for osteogenic proteins (24-26). Although collagen itself is an inadequate bone graft, when combined with ceramics and growth factors, it becomes a powerful inducer of bone regeneration (27, 28).

Scaffolds comprised of synthetic polymers offer many advantages over natural polymers including reproducibility, unlimited supply, relative lack of immunologic concerns, and tailorable properties such as degradation rates and mechanical strength. Synthetic polymers used for bone regeneration include poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactic-co-glycolic) acid (PLGA), polypropylene fumarate (PPF), and the polyhydroxyalkanoates (PHAs) (29). Combining polymers with ceramics creates bioactive scaffolds that enhance tissue formation with greater initial strength.

A common methodology of fabricating ceramic-polymer composite scaffolds is promoting the deposition of a mineral layer on its surface from a solution with ion concentrations similar to that of human plasma (Fig. 1). By immersing PLGA substrates in simulated body fluid (SBF), an *ex vivo* apatite coating comparable to human bone mineral is formed (30, 31). Such

scaffolds demonstrate increased osteoconductivity while maintaining the appropriate porous architecture and degradation kinetics. Expanding on this theme, growth and inductive factors have also been incorporated into similar mineralized matrices with much success (32-34). The deposition of a mineral layer from SBF is a lengthy process, commonly requiring several days. Instead of forming a calcium phosphate layer, a less time-consuming approach involved coating the surface of a VEGF-releasing PLGA scaffold with bioactive glass in order to improve the construct's capacity for bone-tissue maturation (35). Increased angiogenesis was observed in these scaffolds (Fig. 1), which in turn led to greater mineralization of newly formed bone. The results of this study demonstrated that targeting other pathways, for instance vascularization, instead of solely osteogenic differentiation can provide increased benefits. In order to achieve such a multifactorial approach, composites of multiple materials are often required.

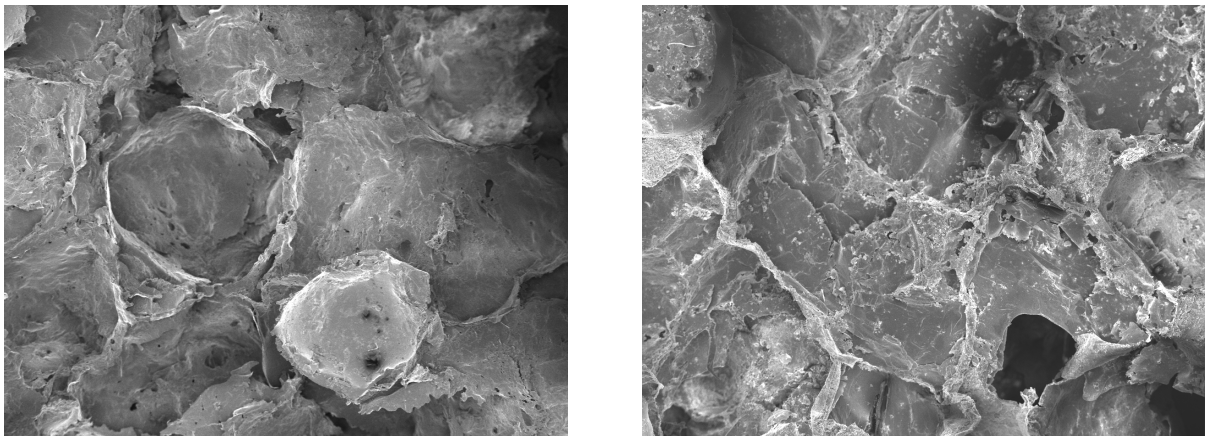

Figure 1. Composites of PLG and two bioceramics. PLGA-hydroxyapatite composites were fabricated by soaking the scaffold in mSBF for 7 d (Left). PLGA-Bioglass composites were produced by submerging the polymeric scaffold in a Bioglass slurry for 5 min (Right). Note that the PLGA-HA composites have smooth pores, while the PLGA-Bioglass composites appear to possess a rough surface.

To further increase cell interaction with bioactive ceramics, composites with nano-sized hydroxyapatite particles are being further investigated (36, 37). Nano-composites allow the inclusion of greater amounts of ceramics that result in enhanced mechanical properties including increased tensile strength, bending strength, impact energy and moduli closer to the order of natural bone while maintaining an interconnective architecture (38, 39). Still, recent studies suggest that the amount of incorporated hydroxyapatite particles plays a lesser role than the distribution within the scaffold achieved by nano-sized particles compared to their macro-sized

alternatives (40). Thus, less hydroxyapatite may be necessary in certain scaffolds depending on the fabrication process. Since hydroxyapatite degrades relatively slowly, smaller initial quantities of the bioceramic will result in less residual material to potentially interfere with newly regenerated tissue. Nano-composite scaffolds were observed to possess short-term suitable biocompatibility and osteoconductivity both *in vitro* and *in vivo* (41). Nevertheless, studies over longer durations are required with different animal models, especially since there is some evidence that nano-hydroxyapatite particles can stimulate human neutrophils to release inflammatory cytokines (42). Thus, the degradation rates of these nano-composite scaffolds may be of increased importance since a sudden release of nano-hydroxyapatite may induce an undesirable immune reaction.

Injectable scaffolds would minimize much of the pain and trauma associated with traditional orthopedic surgeries. The ability to fit the shape of complicated cavity geometries, polymerize *in situ,* and still maintain appropriate bioactivity would potentially give rise to minimally invasive procedures. Research on injectable scaffolds for orthopedic applications is limited, with the two most commonly cited systems based on either poly(propylene fumarates) (PPFs) or polyanhydrides (43-47). Limitations associated with these systems include low mechanical strength and acidic degradation products. A two-component injectable polyurethane system with incorporated β-tricalcium phosphate granules was recently developed in order to address these issues (48). This system demonstrated superior mechanical properties compared to conventional injectable bone scaffolds while preserving proliferation and viability of human osteoblasts *in vitro*. However, no studies on the ability of this system to promote osteogenic differentiation have been conducted nor has this system been tested *in vivo*. Although further examination is necessary, the combined presence of the polyurethanes and the calcium phosphate is a promising alternative to conventional bone grafts.

Other materials besides ceramics can be used in conjunction with a polymer scaffold to increase bone regrowth. The surface of synthetic scaffolds can be coated with natural materials to improve osteoblast adhesion, proliferation, and differentiation (49, 50). This process further removes the inherent hydrophobicity of the construct, thereby potentially increasing osseointegration when implanted. Composites containing carbon nanofibers and nanotubes have exhibited increased osteoblast activity and binding (51-53). Additionally, carbon nanotubes may be functionalized with other bone-inducing substances while drastically improving the

mechanical properties of implants (54). However, these nano-carbon materials are not biodegradable and will remain a permanent fixture in the area of bone regeneration, thereby raising concerns regarding immunogenicity and fibrosis. Although ceramic-polymer constructs comprise the most common tissue engineering approach to induce bone regeneration, there are several other composite technologies currently being explored that possess different, but positive osteogenic benefits.

The field of bone tissue engineering is rapidly developing to meet the needs of clinical medicine. Constructs promoting bone regeneration can be pre-formed or injected and cured at the site of the defect. Materials used to achieve bone regeneration are diverse including but not limited to metals, ceramics, synthetic polymers, naturally derived polymers, and other biocompatible substances. Success has been found by combining these materials as a strategy to eliminate the disadvantages of an individual material. Further studies need to address the defect size limitations of each construct along with the regenerative capabilities of the scaffolds when implanted in different disease scenarios. Much work still needs be completed before tissue engineered constructs challenge autogenous bone grafts as the predominant treatment for bone defects, but the benefits to be obtained from these technologies cannot be overlooked.

3. COMPOSITES IN VASCULAR TISSUE ENGINEERING

With obesity, type II diabetes, hypertension, and other cardiovascular risk factors on the rise in developed countries, vascular systems engineering is gaining a more prominent position in the practice of preventative and restorative medicine (55). The vascular system is responsible for many of the functions regulating physiological homeostasis including supplying nutrients to cells, removing cellular waste, controlling pH and stabilizing body temperature. Disturbances in vascular function are often met with severe consequences. Research in recent years has focused on tissue engineered heart valves (TEHV) and engineered blood vessel substitutes as potential interventional treatments for specific cardiovascular disease pathologies (56-58). By combining a scaffold for physical support, a favorable cell source, and biological signals, constructs are closer to replicating the actions of living native tissues. However, many challenges still exist including but not limited to inappropriate mechanical properties, tissue remodeling, and immune responses.

Composites have been used to counter these issues as interactions of vascular tissues become better understood.

3.1 Tissue Engineered Heart Valves

A substantial fraction of prosthetic heart valves implanted annually in the United States are mechanical, and although durable, they are associated with a substantial risk of thromboembolic complications (59). Hence, bioprosthetic implants such as glutaraldehyde-preserved porcine aortic valves and bovine pericardial valves have become increasingly popular (60). Although these valves do not require the patient to undergo anticoagulation therapy, they often necessitate re-operation due to cuspal calcification leading to structural failure (61). Allografts are considered more biocompatible than xenografts and they display satisfactory hemodynamics; however, donor tissue is in limited supply and they are still subject to calcification (62). Augmenting the need for a tissue engineered valve is the shortage of size-appropriate allografts for pediatric population (63). Additionally, these implants are incapable of adjusting to the rate of patient growth, requiring repeated operations to achieve suitable vascular flow for the child. Tissue engineers are attempting to address these inadequacies by creating constructs that will be capable of functioning, remodeling, and developing in the same manner as native valves (64), yet the fabrication of composite constructs has met with limited success in this field to date.

Valves composed purely of PGA, PLA, or copolymers of both have proven to be too stiff, bulky and rapidly degradable to induce an appropriate extracellular matrix from cells seeded *in vitro* (65). To address these shortcomings, a trileaflet valve composed of a non-woven PGA mesh coated with poly-4-hydroxybutyrate (PH4B) was fabricated, seeded with autologous myofibroblasts and endothelial cells *in vitro*, subjected to increasing pressure and flows by a pulse duplicator system for fourteen days to simulate the vascular environment, and implanted in the pulmonary valve position in a lamb model (66). PH4B, which has a longer degradation time than PGA, was used to maintain the mechanical strength of the valve while allowing seeded cells to benefit from the porous scaffold it enclosed. Constructs examined after implantation for five months displayed similar mechanical properties and cellular layers resembling the elastin, glycosaminoglycans, and collagen layers of native valves. Further studies using this valve construct demonstrated the ability of cells derived from ovine bone marrow to survive and manufacture a tissue with many functional resemblances to native valves. Such constructs have

also exhibited responsiveness to stimulation by soluble signals in the media to improve *in vitro* conditioning of endothelial progenitors (67, 68). A recent approach utilized fibrin to seed the cells on the composite scaffold before the construct underwent mechanical conditioning with a diastolic pulse duplicator, potentially creating a construct strong enough to implant in the aortic valve position (69). Results were mixed as constructs demonstrated enhanced tissue functionality and mechanical properties, but failed to achieve ideal anisotropic properties or closure dynamics. These studies have shown valves fabricated from PGA and PH4B to be promising potential replacements for native tissue, yet further issues need to be addressed such as the long-term effects of these constructs placed *in vivo*, strategies to limit or eliminate an immune reaction, and fabrication techniques to produce valves capable of withstanding the stronger left ventricular pressures naturally occurring in the aortic position.

Scaffolds destined to replace aortic valves must be stronger and more robust than those acceptable for pulmonary valve positions. Mathematical modeling has shown that PGA-PH4B composites demonstrate stiffer, less anisotropic mechanical behavior in conjunction with incomplete coaptation compared to native porcine leaflets when subjected to transvalvular aortic pressure (70). These results combined with the experimental trials mentioned above suggest that PGA-PH4B composite valves may not be suitable for aortic replacement.

Researchers have attempted to fabricate valves comprised of different materials in order to achieve the mechanical properties necessary for aortic valve implants. A knitted, fibrin-covered polycaprolactone valve seeded with myofibroblasts demonstrated proper opening and closing dynamics, good biocompatibility, and increased durability (71). However, the valves also possessed an unacceptable amount of regurgitation and the deposited extracellular matrix was not examined or compared with that of native tissue. Improvements to limit the amount of leakage in the pores and further histological assays need to be performed before these constructs can be considered for *in vivo* use. A different approach used poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3/4HB) to reinforce a decellularized extracellular matrix (72). Results showed that this valve had decreased thrombogenic potential, greater tensile strength, and increased suture retention strength when compared to decellularized matrices alone. Additionally, these constructs remained viable for 12 weeks in a rabbit aorta and demonstrated a complete endothelial layer. Still, *in vivo* studies in more common, larger animal models such as sheep or lamb must be completed, studies of longer duration are needed, and the immunogenic

concerns regarding incomplete removal of cells or cellular debris characteristic of all decellularized xenograft matrices still remain.

Much work still needs to be completed before tissue engineered composite heart valves are implanted in humans. Other tissue engineered approaches were better. For example, human decellularized pulmonary valve allografts reseeded with autologous peripheral mononuclear cells performed well when implanted in the pulmonary valve position of two pediatric patients (73). Throughout the 3.5 year course, these valves functioned appropriately and grew parallel to somatic growth. However the sample size was small and this approach is still limited by the amount of donor tissue and potential for immunogenic concerns if the construct is not sufficiently treated for antigenic material. Composites could eventually eliminate these concerns, but new fabrication techniques to optimize mechanical properties, hemodynamics, and extracellular matrix deposition need to be found.

3.2 Blood Vessels

Coronary artery and peripheral vascular diseases are becoming increasingly prevalent in the United States (74, 75). In current clinical practice, autologous vessels such as the internal mammary vein and the saphenous vein are routinely used for grafting bypass procedures (76). Still, many patients do not possess an appropriate vessel due to multivessel vascular disease, amputation, age, or previous use, and allograft supplies are limited. Thus, there is a need for engineered blood vessel substitutes that can meet the mechanical, biological, and hemocompatibility requirements of native vessels while remaining patent for many years. At their simplest level, vessels serve as a conduit for blood. However, vessels also have more complex functionalities under sympathetic nervous system control. Vessels are capable of rapidly constricting in response to physiological cues, leading to changes in peripheral resistance and ultimately regulating blood flow and tissue perfusion (77). Consequently, elasticity and compliance are key components in the ideal blood vessel construct. Native vessels have an endothelial lining that serves to prevent thrombus formation and leakage. Engineered blood vessels should also have a luminal surface that avoids these undesirable events (78). Small vessels (< 6mm) in particular pose a worry for thrombogenicity since blood flow velocities are lower leading to increased potential of activating the coagulation cascade (79). Additional material considerations are necessary for small diameter vessel replacements. Researchers have

found that the use of composite biomaterials is often essential to match the properties of engineered blood vessels with native tissue.

Constructs composed of expanded polytetrafluoroethylene (ePTFE) have been used clinically for almost thirty years due to the low thrombogenicity potential, porous scaffold nature, and high strength (80). These synthetic vessels, however, are relatively noncompliant constructs leading to a compliance mismatch situation between the engineered and native vessel (81). A series of undesirable events soon follow implantation including intimal hyperplasia, activation of coagulation and complement cascades, thrombus formation from turbulent flow, and finally graft malfunction (82, 83). In order to limit the thrombogenicity of ePTFE constructs, modifications have been made including the addition of synthetic molecules and extracellular matrix materials to promote endothelial cell adhesion and decrease turbulence (84-86). A unique approach to this methodology was the creation of a phospholipid membrane-mimetic film via *in situ* photopolymerization on the luminal surface of a gelatin infused ePTFE graft (87). Compared to uncoated ePTFE grafts, the composite graft was stable under high shear rates and prevented platelet and fibrinogen deposition, a thrombus precursor, during a 1 hour period of blood flow in a baboon model. Additionally, the phospholipid membrane was capable of supporting the attachment of various ligands to promote endothelialization of the graft. Still, researchers are looking for alternatives to ePTFE grafts since the underlying problem of compliance mismatch remains.

In addition to synthetic biomaterials, studies have explored the effectiveness of composite scaffolds fabricated from many naturally occurring materials. Composite scaffolds of collagen and fibrin were found to have superior mechanical properties than scaffolds comprised solely of the pure component alone, and these properties can be further enhanced by altering the concentration ratios of the constituents (88, 89). Previous studies have shown that vascular smooth muscle cells seeded on fibrin gels secrete more elastin than collagen gels (90). Elastin is known to further increase the amount of deformation a construct can withstand, improve the remodeling process, and is essential for withstanding the pulsatile blood flow and recovering from vessel contraction (91). Hence, it is likely that a collagen-fibrin hybrid scaffold would inherit increased mechanical benefits *in vivo*. These concepts were further illuminated in studies characterizing a collagen-elastin vascular graft (92). Not surprisingly, mechanical properties were improved and estimated burst pressures were higher for the composite graft. Vascular grafts

comprised of biological materials have the advantage of forming tissues with architectures that are more similar to native vessels, yet it is widely regarded that they currently do not possess adequate strength for clinical use (93-96). By combining materials, biologically-based scaffolds have experienced a surge in mechanical properties, but the question persists: will it ever be enough?

Tissue engineered blood vessels strive to be a viable alternative to autografts and allografts. However, most bypass surgeries are performed on an urgent basis while, in direct contrast, engineered constructs often require weeks of mechanical conditioning or growth in an *ex vivo* phase to gain the necessary properties of an adequate vessel. Future approaches may need to consider temporal factors if they are to be effectively translated into clinical practice. The appropriate combination of multiple materials may provide the essential initial strength to exist *in vivo*, thus allowing time for the construct to be remodeled and allow the tissue elements to grow and mature.

4. COMPOSITES IN NEURAL TISSUE ENGINEERING

The nervous system's physiology and structure are complex. Designed to receive, decipher, and transmit information throughout the body, it offers a challenge to engineers attempting to replace injured tissue while maintaining the system's multiple modalities. The functional unit of the nervous system, the neuron, is derived from ectoderm and is responsible for the anatomic and trophic organization. Consisting of a body, its processes, dendrites and a solitary axon, this cell has lost its ability to undergo cell division. Neuroglia, however, are capable of mitotic cell division throughout their lifespan, especially in response to trauma (97). Rational regeneration attempts require attention to both these central and environmental features (98, 99). In order for implants to serve as successful treatments, multiple technologies should be included to ensure that all viable components are addressed and can act synergistically to provide maximal reparative benefit. Several materials may need to be combined in conjunction with inductive factors and transplanted cells in order to achieve functional neural tissue recovery.

4.1 Peripheral Nervous System

Axonal regeneration is possible over short distances in the peripheral nervous system, with the amount of regrowth dependent upon numerous factors including the location of the lesion and

the age and health of the individual (100). In the event of total transection of the axon including its myelin sheath and endoneural tube (neurotmesis), a series of complex cellular events involving Schwann cells, macrophages, and monocytes follows rapidly. The severed distal nerve fiber undergoes Wallerian degeneration during which the Schwann cells regulate the destruction of their myelin sheaths (101). Macrophages migrate to the area and are responsible for phagocytosing the resulting debris while also secreting growth inhibitory cytokines (102). Schwann cells proliferate, filling in the void left from the degenerated section. In a coordinated effort, they form the longitudinal cell Bungner bands which direct the regenerating axons. At the proximal end, new axon sprouts are formed and advance toward their targets via physical and chemical mediated signals such as laminin, nerve growth factor (NGF) and neurotrophin 3 (NT-3) (103-105). Those axons that reach their targets establish neural functionality while the others eventually degrade. However, autonomous peripheral nerve regeneration and functional recovery is often disappointing and not applicable to large lesions.

When neurotmesis occurs, two treatment options are currently available in clinical medicine: join the ends of the lesion or fill the void resulting from the lesion. Coaptation, the surgical reuniting of the nerve ends, is usually reserved for short lesions and presents several disadvantages. Sutures can cause an undesirable immune reaction in addition to placing extra tension on the repair site, resulting in worse outcomes (106, 107). Currently, the most common method for repair of peripheral neuropathies is the autologous nerve grafting procedure. Newly regenerated axons of the proximal nerve stump are contact-directed towards their target by the surgically implanted foreign nerve. Shortcomings of this technique include loss of donor site function, donor site morbidity, and the need for multiple surgeries in order to harvest the nerve before it is grafted (108). Additionally, nerve size mismatch, modality disparity, and neuroma formation can complicate recovery. The present standard of care is to use sensory nerve grafts, particularly the sural nerve from the posterolateral side of leg, despite findings that mixed nerves have worse outcomes with this method (109). As a result, functional recovery of neural tissue after implantation of autologous nerve grafts is often inadequate (108, 110).

Researchers have recognized the need for a synthetic alternative to autografts for peripheral nerve regeneration. Much focus has been placed on nerve guidance channels (NGCs) as a potential resource for guiding axonal outgrowth between damaged nerve ends (Fig. 2). These hollow tubes provide space along which to grow with contact guidance for axonal

regeneration. They also allow for communication between the proximal and distal nerve stumps. Studies in humans using NGCs have been met with mixed results. Nonresorbable, biocompatible NGCs comprised of either silicone or polytetrafluoroethylene have demonstrated the capacity to support axonal regeneration (108, 111-113). However, disadvantages of the use of nondegradable artificial nerve guides include inflexibility and compression of regenerated axons resulting in chronic pain and discomfort. Thus, NGCs comprised of biodegradable synthetic materials such as PGA and polylactide-caprolactone are held in higher favor (114-117). However, single-molded NGCs are only accepted for short neuronal defects limited to a few millimeters, as autografts tend to have improved performance for longer gaps.

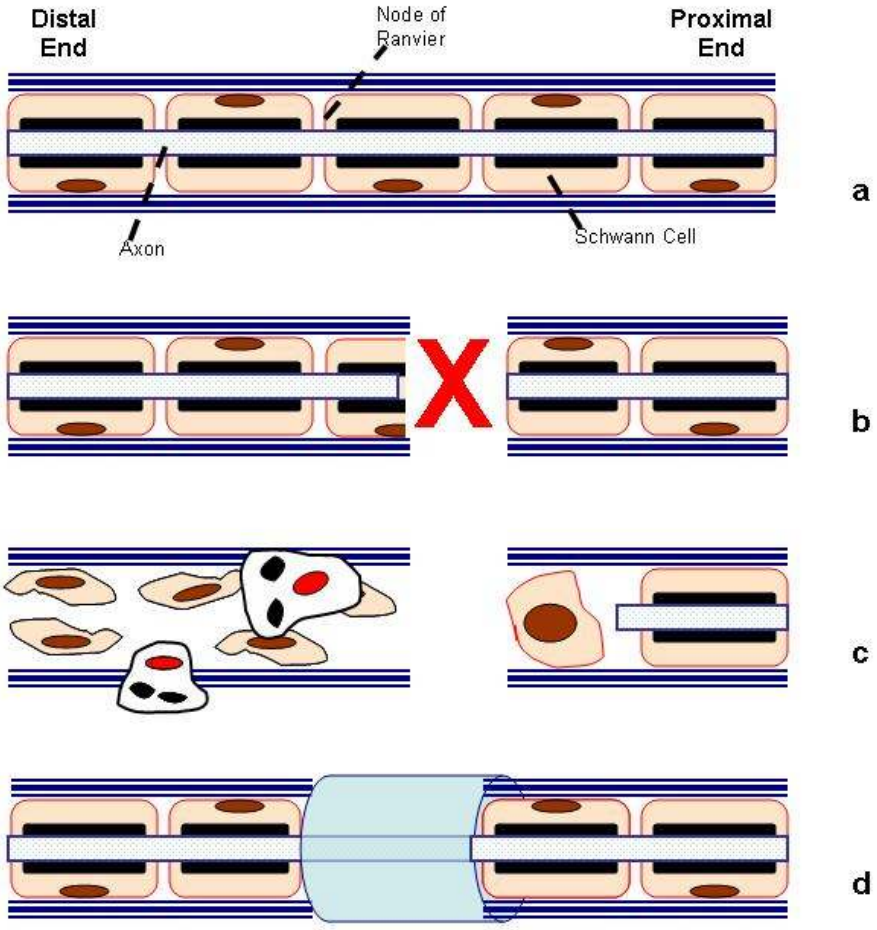

Figure 2. (a) Normal peripheral nerve (b) Neurotmesis (c) Wallerian degeneration (d) Implanted nerve guidance channel

For critical-sized nerve lesions, a simple hollow tube is inadequate for axonal regeneration. Longer defects require engineered constructs that provide increased physical support and biologic activity. Researchers have turned to creating composites with tailorable properties to enhance controlled regeneration across peripheral nerve gaps (118, 119). Approaches are numerous including filling the lumens with natural (collagen, laminin, fibrin) and synthetic fillers (polyamide, polydioxanone, polyglactin) and incorporating various neurotrophins (fibroblast growth factor, glial growth factor, NGF) (117, 120, 121). For instance, guidance channels fabricated from poly(hydroxylethyl methacrylate-co-methylmethacrylate) P(HEMA-co-MMA) hydrogels have been embedded with PLGA microspheres containing a potent neurotrophin (NGF). This strategy has resulted in a nerve conduit, capable of delivering neurotrophins in a sustained manner (122). Not unlike other cell-based therapies in regenerative medicine, the addition of cells which can directly participate or promote tissue formation has resulted in improved neural repair. The enrichment of various constructs with Schwann cells has shown increased promise, likely due to the critical role of this cell type in axonal regeneration (123-125). Multidisciplinary methods have demonstrated that the benefits of single components can be synergistic, and composite conduits may lead to a better outcome for nerve repair.

4.2 Central Nervous System

In contrast to the peripheral nervous system, the central nervous system (CNS) possesses a severely limited ability to regenerate following insult. Cell replacement does occur after injury, but the course is gradual and restricted to the neuroglia of the CNS: the astrocytes and oligodendrocytes (126). Axons are stimulated to grow into the defect but terminate at the lesion site, preventing reinstatement of the neuronal circuitry. Neuronal growth inhibitors are upregulated, and reactive astrocytes form a formidable barrier to axonal regeneration, termed the glial scar (127). The challenge for neural tissue engineers is to provide substrates that allow neuronal infiltration and proliferation in such a hostile environment without compromising the blood-brain barrier or instigating further inflammation.

Cavities in the brain can result from traumatic brain injury, late phase stroke remodeling, or several neurodegenerative diseases. Still, there have been fewer research efforts focused on the development of substrates to fill these voids, and much of this work has focused on the development of gels (128-131). Hyaluronic acid (HA) hydrogels have been used in other tissue

engineering applications such as cartilage engineering and post-operative peritoneal adhesion prevention and have found favor as a scaffolding material for neural tissue (132-135). One approach activated HA hydrogels with 1,1'-carbonyldiimidazole before laminin deposition from a sodium bicarbonate solution. Laminin, a glycoprotein secreted into the extracellular matrix, has demonstrated the capacity to promote neurite outgrowth and axonal regeneration in addition to serving as an axonal guide (105). Compared to autonomous CNS recovery, HA hydrogels and HA-laminin gels showed glial scar reduction, increased integration into the surrounding parenchyma, increased cell infiltration, and increased angiogenesis. However, neurite migration, extension and regrowth were only observed in the HA-laminin gels (136). Photopolymerizable poly(ethylene)glycol (PEG)-based hydrogels have also been explored as neural scaffolds (137). These scaffolds show promise since they are capable of conforming to the shape of the cavity, possibly resulting in increased integration into the cortex. PEG-poly(lactic acid) (PLA) hydrogels were formed with collagen and cells co-encapsulated inside. Alone, these composite gels did not show increased cell survival or metabolic activity over native PEG–PLA gels. When basic fibroblast growth factor-2 (bFGF-2) was added to the media, cell survival and metabolic activity increased relative to native PEG-PLA gels cultured in the bFGF-2 media, suggesting a synergistic interaction between bFGF-2 and collagen (138). PEG-PLA hydrogels were recently constructed with ciliary neurotrophic factor (CNTF) and PLGA microspheres encapsulating NT-3, forming a composite system capable of delivering neurotrophins with separate release profiles (139). Distinct release kinetics can be used to deliver the appropriate molecular signals at the suitable time in neuronal regeneration, reducing waste of growth factors and perhaps providing necessary cues over a more physiological temporal sequence. The use of multiple biomaterials which may be independently manipulated provides yet another dimension of control over substrate properties.

Composites containing single-walled carbon nanotubes (SWNTs) are being investigated as a suitable CNS implant material due to their high mechanical stability, corrosion resistance, and electrical conductivity (140, 141). Films manufactured from poly(diallyldimethylammonium chloride) (PDDA) and layer-by-layer (LBL) assembly of SWNTs and poly(acrylic acid) (PAA) showed increased cell viability of NG108-15 neuroblastoma and glioma hybrid cultured cells than on PDDA or PAA films alone (142). Thin LBL films of poly(ethyleneimine) (PEI) and SWNTs demonstrated no adverse effects on the viability and differentiation of neural stem cells

suggesting this material may be an appropriate choice for neural prosthetic devices (143). The electrical conductivity of LBL PAA/SWNT thin films was used to achieve an electrophysiological response from differentiated NG108-15 cells (144). These studies have shown *in vitro* that SWNTs are not only a biocompatible reinforcing material but may stimulate cells to regain neural functionality when implanted as devices for neural regeneration.

Spinal cord disease and injury often results in permanent disablement below the level of the lesion. The first line of clinical therapy for spinal cord injuries (SCI) is the administration of high doses of methylprednisolone to prevent further neurological deficit caused by inflammation (145). Although treatment with this steroid produces improved functional outcomes, it is insufficient as it offers little hope of substantial neurological recovery. Biomaterials have been developed to promote the recovery of any transected or displaced descending motor or ascending sensory tracts throughout the spinal cord. Numerous natural (collagen, alginate, hyaluronic acid) and synthetic polymers PEG, poly(D,L-lactic-co-glycolic acid, polycarbonate) have been used to manufacture gels, sponges, and tubes for neural tract regeneration in SCI (Fig. 3) (146, 147). Although there are many single biomaterial-based approaches to spinal cord regeneration, composites are relatively limited and are just beginning to gain notice. Recently, copper-capillary alginate gels (CCAGs) with a linear microtubular structure have been complexed with oligochitosan to prevent dissolution (148). These gels showed biocompatibility with mouse embryonic stem cells and were capable of inducing long cylindrical cellular structures within the microtubules. Possibly with the addition of cellular cues to gain further differentiation, this gel can be applied to neural tissue engineering as a means of spinal cord axonal regeneration.

These studies demonstrate the potential benefits of a combinatorial approach towards neuronal and neuroglia regrowth. However, more research is necessary to determine the optimal cell source, the role of inflammatory factors on these constructs, and their mechanical properties. Additionally, these constructs are often placed in hypoxic environments resulting from injury and thus, the role of oxygen concentration on the regenerative effects of the construct should be further explored.

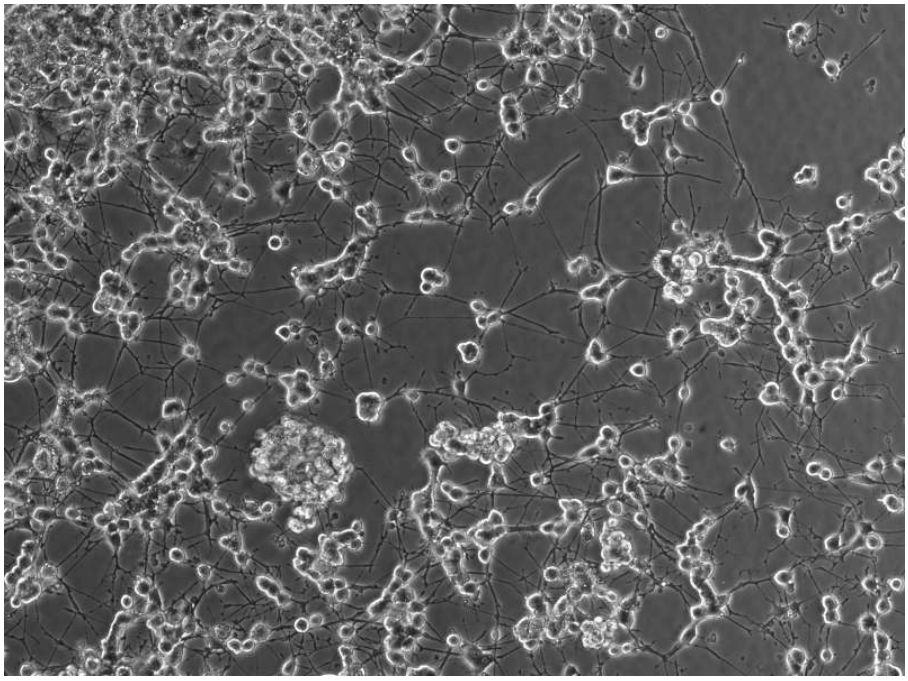

Figure 3. PC12 cells cultured on a collagen substrate demonstrating the formation of a two-dimensional neural network and axonal growth.

5. CONCLUSION

Composites have gained prevalence in the field of tissue engineering due to the lack of individual biomaterials satisfying the multifunctional needs of regenerating tissue. Numerous successful applications exist in bone and neurological systems where the beneficial properties of each individual component of composite systems act synergistically when combined, demonstrated by enhanced bioactivity and increased integration into host tissue. Composites in vascular systems, particularly heart valve replacements, possess more limited positive outcomes as the deficiencies of each material are compounded, currently outweighing any additive gains. However these inadequacies may be eliminated as new biomaterials are discovered, cell sources are optimized, and delivery systems are better developed.

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