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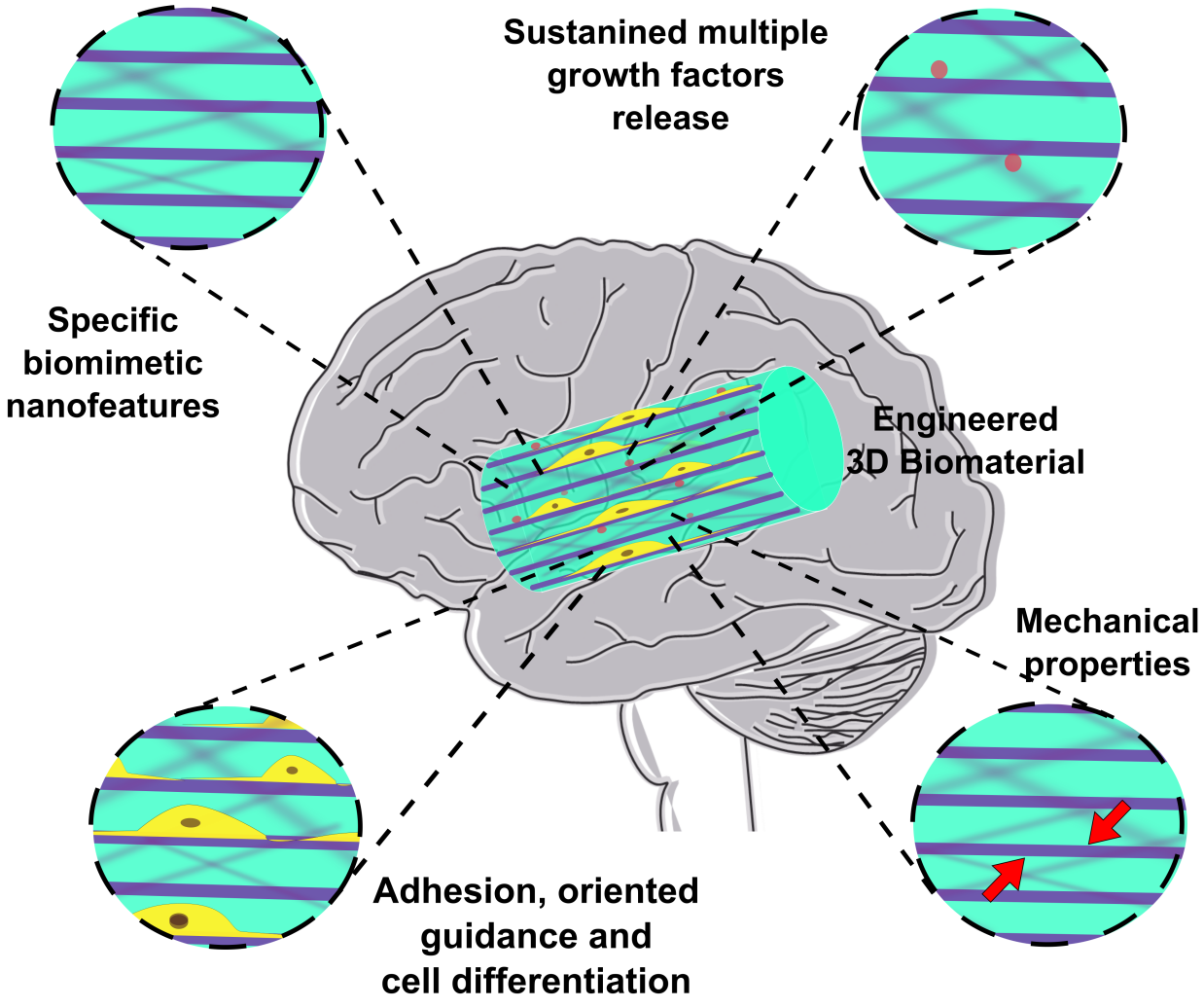
3D Biomaterials as a model for neural tissue microenvironment



Journal Pre-proof

Biomimetic materials and their utility in modeling the 3-dimensional neural environment

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Summary

The brain is a complex 3-dimensional structure, the organisation of which provides a local environment that directly influences the survival, proliferation, differentiation, migration and plasticity of neurons. In order to probe the effects of damage and disease on these cells, a synthetic environment is needed. Three-dimensional culturing of stem cells, neural progenitors and neurons within fabricated biomaterials has demonstrated superior biomimetic properties over conventional 2-dimensional cultureware; offering direct recapitulation of both cell-cell, as well as cell-extracellular matrix interactions. Within this review we address the benefits of deploying biomaterials as advanced cell culture tools capable of influencing neuronal fate and as *in vitro* models of the native *in vivo* microenvironment. We highlight recent and promising biomaterials approaches towards understanding neural network and their function relevant to neurodevelopment and provide our prospective on how these materials can be engineered and programmed to study both the healthy and diseased nervous system.

Introduction

The brain is the most powerful and complicated organ in the human body, yet remains the least understood. The USA and the European Union recently launched major research programs that will focus on enhancing our fundamental understanding of the brain and its development (Amunts et al., 2016). These studies have recognised our limited understanding of the tightly orchestrated sequences of events underpinning neurodevelopment is the direct reason for inadequate treatments options available for various types of neural injuries or diseases such as stroke, trauma or neurodegenerative disorders. Therefore, neurodevelopmental research is evolving to develop new methodologies for *in vitro* 3D culture of neural tissue, such as brain organoids, allowing a benchtop model of the intricate *in vivo* structure, and to utilize these to advance our understanding of the development and function of the human brain (Hogberg et al., 2013). Significant advances in the engineering of intelligent, programmable and above all, organisationally fluid microenvironments will allow, for example, the support and study of brain organoids over time and disease like conditions. By effectively modelling the brain as it recovers and responds to injury and damage, we can unlock new and vital understanding to enable the development of advanced treatments for neural repair (Orive et al., 2009b, Mitrousis et al., 2018).

Over recent decades, the field of biomaterials science has made significant progress in developing biocompatible materials that are able to mimic aspects of the complex features of the *in situ* cellular microenvironment. In the past, research has predominantly cultured cells in under conditions poorly matched to the physiological milieu – such as two-dimensional (2D) plasticware thatoffers only hard, unchanging surfaces that are unable to replicate the tightly orchestrated sequence of genetic, environmental, biochemical, and physical events present during neurodevelopment (Tibbitt and Anseth, 2009, Ravi et al., 2015). As a result, cells cultured in 2D environments typically exhibit irregular and unnatural responses; giving unreliable information on morphology, gene expression, cellular functions, and cell-cell interactions to name a few (Carletti et al., 2011). Although there is no doubt that traditional 2D culturing systems retain a place and have been useful for some cell-based studies to increase our knowledge of basic cell biology (Antoni et al., 2015), it is now recognized that there is a crucial loss of the *in vivo* tissue-specific architecture (Birgersdotter et al., 2005).

Within the *in vivo* tissue microenvironment, cells exist in a connected state; both to each other as well as to a dynamic extracellular matrix (ECM). This structure forms a supportive and fibrous three-dimensional (3D) structure, which, as it is cellularly excreted, can also be continuously remodeled by its population of cells in response to the state of the tissue. It presents an information rich, specific ordering of a variety of proteins such as laminin, fibronectin, elastin and collagen and provides biochemical and mechanical signals, with its precise composition having implications upon cell phenotype (Lau et al., 2013). In contrast to 2D cell culture systems, where it is impossible to mimic many of the crucial biological properties of the *in situ* milieu, 3D culture systems are capable of recapitulating components of the natural 3D ECM structure, allowing cells to proliferate, differentiate, migrate, and communicate along analogous pathways to those within native tissue (Baharvand et al., 2006). At present, numerous biomaterials in different formats are being optimized as scaffolds to support standard neural culture in 3D or cerebral organoids. Programmable scaffolds that can be mechanically, biochemically and topologically tuned are being bioengineered to produce the next generation of brain organoid models, where scientists can select molecular, cellular, and structural features reminiscent of the native human brain. These materials will increasingly enable researchers to predictably programme the attachment, proliferation, and differentiation of progenitor cells *in vitro*, making it possible to probe complex events such as neuro-glia interactions and neurocircuitry construction (Greiner et al., 2012).

To date the majority of review articles involving biomaterials for neural tissue regeneration focus on the deployment of different scaffolds for neural cells, methods of fabrication, and properties. As such, the interested reader is directed to the excellent review available on these topics (Orive et al., 2009a, Pettikiriarachchi et al., 2010, Tuladhar and Shoichet, 2018, Paul Donald Dalton and Mey, 2009). However, there is a gap surrounding the biological interactions between neural stem/progenitor cells and the various biomaterials and their deployment as 3D culture tools for the *in vitro* development of neural tissue models. This is largely due to the fact that the application of biomaterials in studying neurodevelopmental is relatively limited, particularly in the context of directed neuronal differentiation of pluripotent stem cells. Encouragingly, the outlook is optimistic as many of the lessons learnt from the engineering of biomaterials to promote neural regeneration (Wang et al., 2012b), can be adapted to study neural development as well as adult neurogenesis. Here, we provide a

review with emphasis on the importance of the cell-biomaterial interaction that aids in the generation of biomaterial-based tools to advance our understanding neural tissue development, function and dynamics. We provide a brief overview of basic cellular and functional processes relevant to neurodevelopment, before emphasizing where and how 3D biomaterials will enhance our ability to recapitulate key aspects, and ultimately lead to the advancement of new approaches for modelling of both healthy and diseased neural tissue.

Neural stem cells, their niches and the extracellular matrix: a dynamic network

The remarkable complexity of our brain lies in the vast array of cell types that are generated from a small pool of neural progenitors that are subjected to several divisions during development. Regulated by intrinsic and extrinsic cues, these neural progenitors are subjected to transcriptional changes that facilitate lineage specific differentiation to distinct cell fates (Kohwi and Doe, 2013, Pearson and Doe, 2004).

In the developing embryo, cell division and migration are critical to organize embryonic neuroepithelial cells (NECs) into mature neurons and glia cells. To begin, neuroepithelial cells divide symmetrically within the ventricular zone (VZ) producing two identical multipotent daughter cells in an orientation known as apical-basal polarity, extending from the ventricular (apical) to pial (basal) lamina (**Figure 1**) (Arai and Taverna, 2017). Subsequently, neural stem and progenitors cells subdivide in a secondary germinal area above the VZ, the subventricular zone (SVZ).

Similar active neurogenic zones are also present within the adult brain: most heavily studied are the SVZ on the walls of the later ventricle (**Figure 2**) and the subgranular zone (SGZ) layer of the dentate gyrus in the hippocampus, (Seri et al., 2004, Quinones-Hinojosa et al., 2006). A number of other neurogeneic niches have also been described, yet less stringently studied and validated.

Efforts to replicate features of the embryonic and adult neural stem cell niches remain a goal for the field, striving to promote organized neurogenesis and guided circuity reconstructions to treat a raft of brain injuries and disease. Thus, one approach to advance the treatment options for patients would be to engineer programmable biomaterials that replicate features of neurogenic niche inclusive of defined VZ and SVZ-like regions.

The niche is a specialised and dynamic microenvironment which is made up by stem cells and a set of other cells that provide a combination of intrinsic signals and specific extracellular conditions (extrinsic determinants). The niche also protects stem cells from gene mutations that might lead to malignant transformation (Greco and Guo, 2010). In the adult nervous system, the niche maintains stem cells in their quiescent state, but after injury, the microenvironment actively signals to the stem cells promoting either their self-renewal or their differentiation to promote tissue repair (Seri et al., 2004). The role of the niche during embryogenesis is different. It produces a variety of factors that act on the stem cells to alter their gene expression, promoting the proliferation and differentiation necessary for development of the fetus. This dynamic function of the niche and the role of the ECM during development are critical to move towards more advanced *in vitro* 3D tissue models to better understand neurodevelopment functions and networks.

Although embryonic and adult neural stem cell niches have been the focus of extensive investigations, several regulatory mechanisms that allow stem cells to meet the physiological demands remain unknown, which is largely due to our current inability to spatial and temporal deliver the necessary physical and biochemical features with traditional culture systems. To effectively replicate these features, a logical method is to base these materials on the biochemical and morphological features of the extracellular matrix (ECM) as juvenile cells transition toward healthy, functional adult cells. As in all organs, neural cells are closely linked with each other and distributed within the ECM forming an intricate network (**Figure 3**). The ECM is secreted by cells and surrounds them in tissues. Whilst once thought of as merely providing passive, mechanical support for cells, it is now recognised as a highly complex and dynamic scaffold that consists of a raft of biologically active molecules that are tightly regulated and essential for determining the action and fate of the cells that it surrounds. In the brain, the biochemical support includes the regulation of neural stem cell proliferation, migration and differentiation during development and within the adult neurogenic niches (Kazanis and ffrench-Constant, 2011). For more information about the stem cell niche, in particular the role of the ECM, the interested reader is referred to the following concise review (Scadden, 2006).

It is important to consider that physical cell-ECM interactions are capable of influencing cells on a molecular, chemical, or genetic level (Engler et al., 2006, Jang et al., 2010, Yoo et al., 2015, Ma et al., 2008, Wang et al., 2018). In this regard, engineering scaffolds that mimic

many of the features of the native ECM is critical to gaining an increased level of control over stem cell differentiation and development. Importantly, the capacity to engineer artificial ECM mimics by controlling the nanotopography, mechanical properties, and surface biofunctionalisation will further enhance our ability to control cell behavior and improve the regulation of cell fate in bio-artificial scaffolds (Chen et al., 2014). We are now at an interesting stage of history where the importance of multidisciplinary approaches are paramount to engineering artificial tissue substitutes for modelling neural tissue and understanding the adaptive and dynamic process of brain development from the molecular level such as gene expression through to the influence of environmental stimuli.

In addition to physical interactions, the ECM is also capable of regulating activity via the transient or persistent presentation of different growth factors, allowing orchestration of their bioactivity (Gattazzo et al., 2014). This characteristic of the ECM, of acting as a protein "reservoir" that can release or retain soluble biological factors with spatial and temporal control, represents one of the most essential features of the ECM in the dynamics of stem cell niches (Hynes, 2009). Common growth factors, such as epidermal and fibroblast growth factors (EGF and FGF), are crucial niche proteins, and are tools that have found routine usefor the *in vitro* culturing of NPCs (Zheng et al., 2004, Morrison et al., 1987). There has been significant research focus on reproducing an ECM level of control of such growth factors within 3D biomaterial scaffolds. Interactions of growth factors with NSCs have been studied using scaffolds with immobilized growth factors or using microparticles to release growth factors (Mahoney and Saltzman, 2001). Recent studies have been conducted to investigate how the growth factors linked to innovative 3D culture approaches are required to address these challenges (Langhans, 2018). Convincingly, bioengineered scaffolds offer improved tools to allow a better understanding of the ECM-neural stem cell network.

Biological interactions between neural stem cells and materials

Soluble factors play an important role in directing stem cell behaviors within the niche. However, many cellular processes are also influenced by mechanical and biophysical interactions with non-soluble components of the ECM. For instance, stem cell behavior depends on tissue stiffness, which is partially regulated by the ECM composition and organization. Stem cells balance external forces and the mechanical properties of their environments. To achieve this, cells control and stretch their cytoskeleton, generating internal

stress that is transmitted to the surrounding environment. The focal adhesion complex connects the cellular cytoskeleton with the ECM, and thereby helps cells to react to forces generated from the ECM, establishing a mechanosensory system (DuFort et al., 2011). The cellular response to mechanical stimuli is described as mechanotransduction, which includes several pathways with specific transcriptional factors. Moreover, other important effects such as substrate topography and ligand presentation play a vital role in these cellular processes (Lutolf et al., 2009). Especially during neurogenesis, cytoskeletal rearrangement and interaction with the extracellular environment, particularly with extracellular matrix ligands are fundamental. Here, integrins play an important role in binding the ECM components. They mediate the bidirectional signaling activating the direct mechanotransductive signaling and the indirect molecular cascades that regulate the gene expression and ultimately growth and differentiation (McNamara et al., 2010). Hence, biomaterial substrates can be engineered with specific nanoscale features in order to direct specialized behaviors in neural stem cells, which can improve knowledge of neural development and disease outcomes (Nisbet et al., 2009). Since cell-ECM interactions differ considerably between 2D and 3D systems, understanding their influence especially in 3D models, over normal and pathological responses is crucial to help further understanding of healthy neural tissue and to translate such knowledge into medical therapies for treating neural diseases (Walters and Gentleman, 2015).

The basement membrane of the ECM is considered as a dynamic and versatile structure able to regulate cellular behaviours. Physiologically, it presents as a hierarchical nanofibrous composition suggesting the importance of substrate topography. Reflective of this, several studies have focused on the use of electrospun materials to mimic the in vivo nanofibrous morphology, demonstrating that physical structures are major regulators of cell behavior (**Figure 4**). For instance, fiber diameter of laminin-coated electrospun polyethersulfone (PES) mesh significantly control the NSCs differentiation and proliferation (Christopherson et al., 2009). In this study, it was shown through a well-defined series of electrospun nanofibrous scaffolds, that fiber diameter was able to successfully control cell behavior, leading to differences in the lineage specific differentiation and proliferation of NSCs cultured on the different variants. A decreasing trend in proliferation corresponded with increasing fiber diameter, demonstrating that cellular cytoskeletal rearrangement controls and enhances the cellular proliferation. Interestingly, Christopherson et al. showed that there was a link between adhesion, migratory activity and cell differentiation specification. Cells on

283 nm fibers assumed glial cell morphology spreading randomly along the fibrous network, while cells cultured on comparatively larger fibers (749 nm) adopted neuronal specification. These findings clearly suggest that cells are capable of altering their morphology and cell shape in response to a valid communication between the biomaterial and the cell. Correctly done, these synthetic ,external morphological stimuli are able to induce valid intracellular signaling to influence a cell's lineage and proliferative potential. We hypothesise that this combination of physical and chemical signals will lead to 'on-demand' post-translation modifications, principally phosphorylation, where scaffold signals are effectively transmitted to the nucleus to promote transcriptional modifications. In it's simplest form, once cells are attached to the scaffold, mechanotransduction signaling pathways allow the cytoskeleton to communicate to the nucleoskeleton (since they are directly linked via bridging proteins (Haque et al., 2006))therebyresult in chromosomal redistribution with the potential to affect gene transcription (Berger, 2007). Alignment of polycaprolactone (PCL) nanofibers influence morphology, proliferation and neural differentiation capabilities of embryonic and adult neural stem cells (ANSCs), providing a mechanism by which topography can influence stem cells differentiation (Lim et al., 2010a). Here, it was demonstrated that ANSCs respond differently to either aligned or random fibrous substrates; in fact, culturing ANSCs onto aligned fibers significantly enhances the neural fate specification as compared to random fibres. Furthermore, it was shown that neural differentiation has a fiber size dependency, with the highest portion of neural differentiation observed at 480 nm. Based on these results, the fiber topography and alignment drive the cell lineage specification through altering the cell-substrate contacts which results in a specific intracellular transduction signaling. This lead to effective changes in gene expression via cytoskeletal and nuclear distortion influencing factors bioavailability and subsequently cell internal dynamics. Moreover, polyphenylene sulfone (PPSu) scaffolds with different topography (random or aligned electrospun nanofibers) have different effects on the activity of neural stem cells (Hajiali et al., 2018). Aligned nanofibers enhanced axonal growth and extensions enabling higher cellular activity (calcium activity) clearly indicating the effect of the scaffolds in creating a better neural network compared to the normal 2D control. Curiously, this demonstrated the possibility of using fiber alignment to provide direction cues for axons and indirectly neural signals allowing for a better understanding of neural tissue network. Also, it has recently been shown that different diameter electrospun PCL fiber mats scaffolds that mimic the anatomical features presented during neural development (fibers with dimensions similar to radial glia, ca. 1μm and fibers with dimensions similar to small vessels ca. 10μm) result in differential

NSCs migratory responses and morphological reactions (Czeisler et al., 2016). Specifically, neurospheres plated on small fiber mats displayed a migratory morphology and extended processes; instead neurospheres onto larger fiber mats showed spherical morphology and no morphological reactions with minimal interaction to the fibers. This study demonstrated that by resembling the natural distribution of fibers in the developing brain, scaffold surface topography regulates and modulates the migration of neural precursors to their target when specific physical scaffold is preserved. The authors hypothesized that the mechanical properties are detected by cells through the integrin-talin-vinculin pathway. Vinculin is part of the focal adhesion proteins and governs the transmission of force from the extracellular environment to the cytoskeleton through the talin-integrin complex and its activation is triggered by exposure to areas of high forces (Carisey et al., 2013). Therefore, this conclusion presumes that topography itself might induce different migration phenotypes and morphological changes.

In addition to electrospun scaffolds, Beduer et al. developed a compressible scaffold for minimally invasive delivery within the brain tissue (Beduer et al., 2015). They used a cryogel system that facilitated extended neuronal network development from primary cells. To improve the cell seeding, attachment and spreading on the cryogel, the architectural parameters (in this case, pore volume, size and interconnectivity) and adhesive motives (a combinantion of poly-L-ornithine and laminate) were employed to optimize adhesion, cell spreading and most importantly, neurite extension. In fact, this study provided evidence of neurites following the gel walls and bridging small gaps. Furthermore, when cells were cultured in higher cell density, well-formed multi-layered structures were formed. This suggests the importance of the equilibrium between cell-matrix and cell-cell interactions to recreate the natural neural tissue and to allow neural tissue formation.

In summary, the physical properties of the stem cell niche microenvironment certainly influence the stem cell fate and future studies should be undertaken directed at clarifying the intermediate steps that are connecting the intracellular changes and signaling pathways in response to external topographical cues.

As mentioned, cells are able to modify their focal adhesion in response to changes in the physical and biochemical properties of the macromolecular components forming their surrounding matrices. For this reason, integrins and integrin-binding peptides, inspired by, and arising from these functional macromolecules, are important inclusions within biomaterials to promote cell/biomaterials interactions that are functional and relevant to a

particular cellular microenvironment. In fact, current research has focused on the identification and characterization of supramolecular structures presented in the ECM (such as the proteins laminin, and fibronectin) to modulate and regulate signals associated with neurogenesis in a manner dependent on their specificity, concentration and presentation modality (Wojcik-Stanaszek et al., 2011). Fully synthetic and therefore fully characterised peptide epitopes, such as those in scaffolds arising from the self-assembly of peptides (SAPs), have been used in place of animal derived or recombinant proteins or protein fragments (Nisbet and Williams, 2012). The specific spatial conformation of ligands in native macromolecules drives their secondary and tertiary structures, promoting binding to receptors and thus influencing the downstream stem cell responses. Particular peptide sequences, or epitopes, from two key ECM proteins, laminin (IKVAV and YIGSR) and fibronectin (RGD and PHSRN), have been shown to promote neurite outgrowth, neuron differentiation, and cell adhesion (Rodriguez et al., 2013, Zhang et al., 2010, Cheng et al., 2013, Horgan et al., 2016, Aye et al., 2018). Interestingly, stem cells are sensitive to not only the presence of specific peptide epitopes but also to the peptide spacing (in terms of the frequency of presentation of a peptide sequence) and peptide affinity (Kilian and Mrksich, 2012). Consequently, the fate of stem cells can be influenced by modifying the affinity and density of peptides at the cell-biomaterial interface (Kilian and Mrksich, 2012). Recently, Stukel et al., demonstrated that peptide concentration and affinity (varied between linear and cyclic variants of the peptide segment), as well as scaffold stiffness altered cell adhesion. Added to this, peptide concentration influenced cellular differentiation, neurite extension, morphology, and focal adhesion assembly (Stukel and Willits, 2018).

As well as incorporated peptide epitopes, functional ligands in a soluble form, particularly neurotrophins, are extremely important in regulating the neural development. These proteins activate two different classes of receptors, the Trk and TNF families, that regulate neural survival, cell fate and expression of proteins crucial for normal neural function and connectivity (Huang and Reichardt, 2001, Horne et al., 2010, Wang et al., 2016). Consequently, designing materials based on biologically relevant peptides sequences with the potential of incorporating functional proteins into biomaterials has been explored (Elliott Donaghue et al., 2014, Kim et al., 2012). However, current approaches for sustained delivery from materials still present their own problems including incapacity to control temporal and spatial delivery. For instance, SAPs provide an appropriately biomimetic scaffold substrate thanks to the ability to present a high density of functional peptide epitopes (**Figure 5**), and

the capacity to stabilise and deliver multiple native form neurothrophic factors (e.g. GDNF and BDNF) (Rodriguez et al., 2014, Wang et al., 2016, Rodriguez et al., 2018, Nisbet et al., 2018, Bruggeman et al., 2016). Moreover, the incorporation of neurotrophic factor loaded in electrospun materials with SAPs has allowed distinct temporal control over the presentation of multiple factors (Bruggeman et al., 2017). Newland et al. show that cryogel microcarriers consisting of star-shape PEG and heparin are able to be loaded with growth factors. They found that these highly macroporous systems were suitable for neural cell culture and were able to improve cell survival during injection though a cannula, and present a sustained release particularly of GDNF, which they attributed to the negative charge of heparin (Newland et al., 2015b). Furthermore, star-shape PEG-heparin-based hydrogels have been using to investigate how NSCs loose the regenerative capacity in Alzheimer's disease (Papadimitriou et al., 2018). Taken together, these scaffolds represent novel growth factor delivery systems important for advancing neural tissue modelling and polymer therapeutic research for neurodegenerative diseases (Newland et al., 2015a). The capacity to either delay or burst the release of neurothrophins will lead to design of programmable materials according to specific timing at which each protein is required to regulate the cellular processes of a desired cell population.

Biomaterials to study neural development

The issues associated with traditional 2D cultureware are accepted: the resulting cells do not accurately mimic *in vivo* characteristics and behaviour, making for inaccurate *in vitro* modelling. 3D cultures and *in vivo* cell behaviour can be achieved in scaffold-free self-assembled aggregate cultures including neural spheroids. Recent advancements have been made with neural spheroids, successfully achieving intercommunication between neural spheroids of multiple cell types (Birey et al., 2017) but neural spheroids and other scaffold-free 3D culture techniques are still size limited, with larger sized cultures experiencing issues such as poor nutrient and oxygen diffusion to interior cells (Ko and Frampton, 2016, Shuler and Hickman, 2014). Tissue engineering scaffold are typically made from porous materials and can therefore provide physical and trophic support to larger cultures, making biomaterial scaffolds an attractive neurological *in vitro* modelling option. Using a directional collagen scaffold material Odawara et al were able to produce a multilayer 3D neuronal culture to mimic the layered cerebral cortex, and observed interlayer synchronous firing (Lim et al., 2010b). Tang-Schomer et al used a layered silk and collagen composite to create an *in vitro*

brain tissue model from primary cortical neurons with controlled regions of grey (neuron rich) and white (axon only) matter mimetic of the cerebral cortex. Cells were maintained for months and they were able to biochemically and electrophysiologically model homeostasis and traumatic brain injury (Kirik et al., 2017, Place et al., 2009).

Another issue with traditional cell culture is that it can be awkward to artificially generate appropriate disease model conditions in scaffold-free cell culture. For instance, recreating the aged condition that results from exposures to reactive oxidative species (ROSs) is important to accurately model adult onset diseases without inaccurately high levels of resilience as found in induced stem cells, and in culture this has required the introduction of progeria genes to accelerate aging (Campos et al., 2014). On the other hand, biomaterial scaffolds for *in vitro* culture provide a base for prolonged trophic control of the cellular environment to specifically induce required conditions. For instance, laminins are known to influence stem cell maintenance, survival, differentiation, and plasticity (Theocharidis et al., 2014), and have been used to functionalise alginate hydrogel to prolong the viability of co-cultured neural cells (astroglioma, astrocytes, microglia, and neurons) in 3D cell culture (Ibraheem et al., 2014), MMP-9 is an endopeptidase enzyme capable of acting on ECM proteins including collagen and laminin. Developmentally, its presence in the brain is reduced in adulthood, and is associated with increased neural plasticity. In biomaterials, MMP-degradable crosslinking in hydrogels has been used to engineer scaffolds to be invaded by cells secreting MMP (Lutolf et al., 2003).

Biomaterials also allow for the study of cell behavior that might not otherwise be possible. Some normally non-biological materials have been used to study neuron signaling pathways. For example, neurons have been shown to grow readily and display high signal synchronization on nanocarbon structures (nanotubes) (Bosi et al., 2015, Crystal, 2014). Neurons have also been grown and studied on semiconducting indium phosphate nanowire arrays to elucidate the role of nontopographic in their growth and interneuron signaling patterns. Neuron cultures were grown successfully and exhibited synchronized activity, indicating successful intercellular communication (Gautam et al., 2017). In a different material, more mimetic of the *in vivo* brain, functionalization of a laminin-derived peptide hydrogel with the anti-inflammatory molecule, fucoidan, allowed phenotypic control of astrocytes between reactive and cytotrophic states (Lee et al., 2017). Previously, *in vitro*

treatment of astrocytes has been almost exclusively of reactive astrocytes due to the difficulties in achieving the cytotropic *in vivo* state *in vitro*. In this regard, this specific biomaterial scaffold has opened the door to *in vitro* investigation of the roles and behaviours of astrocytes *in vivo.*

While biomaterials scaffolds are used and explored extensively as therapeutic aids, their potential as *in vitro* modelling tools is largely unrealized. By way of example, investigation of the effects of trophic factors and regulation *in vivo* can involve specific cell transfection via viral vectors, (Drury and Mooney, 2003, Domanskyi et al., 2015, Allen et al., 2013) and biomaterial scaffolds can be used to improve viral vector delivery in the brain (Webber et al., 2016, Mitrousis et al., 2018), yet the two fields remain largely disconnected. It is generally accepted that in vivo investigation is more accurate, with efforts to improve 2D culture procedures focused on creating a more *in vivo* like environment. Ko and Frampton, when discussing the progression from 2D to 3D neural cell culture, proposed that it can be a difficult switch for researchers to make despite the demonstrated advantages because the extra complexity of experimental procedures/equipment involved (Ko and Frampton, 2016). The reverse may be true when considering the use of 3D neural cell culture as a modelling tool compared to expensive in vivo studies. These scaffolds have demonstrated the ability to form large scale and long term *in vivo* like models of brain tissue. They have been adopted in regenerative medicine for their ability to promote *in vivo* like tissue regeneration, and could provide this same benefit, along with high throughput testing and customization in the area of *in vitro* modelling as well. The area where the most progress has been made to date is in the deployment of scaffold to study the biological interactions between neural stem cells and materials. Certainly, materials have been structurally, chemically and mechanically optimized to control stem cell behavior, which is a step in the right direction to the realization of biomaterials as advanced *in vitro* modeling tools.

Biomaterials for neural tissue engineering

While the application of biomaterials in neurodevelopmental studies has been relatively limited, significant attention has been paid to their deployment in neural regeneration. Much of the work into repair has concentrated on developing biomaterials to replicate some of the features of either the neurogenic niche or the brain ECM, and as such has inadvertently impacted our understanding of how stem cells, neural progenitors, and neurons interface and

respond to biomaterials. Many classes of biomaterials (inclusive of electrospun, hydrogels, and self-assembling peptides) have been used to either support neural progenitor cells or neurons, or to guide axonal projections. While the focus of this work is regeneration, the potential for the engineered biomaterials to be adapted as *in vitro* modelling tools is significant and, in our opinion, a logical step. Therefore a brief summary of the significant advances made in functionalising scaffolds to provide optimal *in vitro* and *in vivo* properties, with tuneable temporal and spatial delivery for repair, is warranted as a platform for their future translation towards the development of advanced *in vitro* devices to unpick the complexity of neural development and move towards understanding how brain cells interact (discussed in the concluding future direction section).

To develop 3D cell culture methods, scientists have engineered and functionalised artificial materials employing a combination of nanotechnology, material science, biology and regenerative medicine methods. Tissue engineering approaches involve three key elements: a scaffold as a microenvironment to permit cell adhesion, proliferation, migration and differentiation; a specific cell-type; and biomolecules and/or drugs to held and guide cell development and function. Several smart biomaterials have been applied in different tissue engineering fields; the most studied areas include bone, cartilage, muscle formation, skin repair and neural regeneration. In all of these applications the requirement of a biomimetic 3D culture environment has become clear. For instance, therapies for skin grafting have shown that the dimensional aspect of the environment is a crucial fate determinant, whereas monolayer cell culturing systems drive abnormal cell function (Berthiaume et al., 2011, Baker and Chen, 2012). In neural tissue engineering, this requirement has been demonstrated in a variety of ways, particularly where cell based therapies have been combined with 3D scaffolds as a delivery vehicle to promote repair and reconstruction within the CNS (Martino and Pluchino, 2006, Aboody et al., 2011, Lindvall and Kokaia, 2006). A brief timeline of neural stem cells and biomaterials major discoveries is shown in **Figure 6**. However, many challenge remain to promote repair and reconstruction within the CNS, including establishing reliable techniques to guide the stem cell differentiation into specialized neural cells (Shah et al., 2016). We have now reached a cross road where it is essential to utilize and adapt the past advances in biomaterials science to grain a fundamental understanding of neurodevelopment that will ultimately lead to an increased ability to precisely control neural progenitor cell behavior. Currently, we have necessary tools to promote stem cell-based regeneration with some success being demonstrated via biomaterial mediated delivery of soluble factors;

patterned topographies to direct the neural growth and surface functionalization to promote superior cell-ECM interactions (**Figure 7**). We propose that the field is now well positioned to begin to deploy these advanced materials to understand the complexity of neural development and develop *in vitro* models of disease and injury. We have recently demonstrated the efficacy of this approach using programmable nanomaterials to control brain inflammation post traumatic injury. Maclean et al. demonstrate for the first time the development of a 3D culture system capable of controlling the cytoskeletal reorganization of brain tissue. The incorporation of fucoidan (anti-inflammatory and anti-proliferative polysaccharide) in the SAP system (Fmoc-DIKVAV) allowed to study the responses of traumatic brain injury both *in vitro* and *in vivo* (Maclean et al., 2018). Importantly, this system was then exploited to gain novel insights about the brain inflammatory cascade that would be impossible to obtain through *in vivo* experimentation. This work was developed on the back of our research focused on neural repair following stroke (Somaa et al., 2017, Nisbet et al., 2018), highlighting how the rapid advances in biomaterials developed for neural repair can be easily adapted to gain fundamental insights about the brain that will ultimately contribute towards superior outcomes for the neural regeneration field.

Biomaterials, including natural and synthetic materials, that possess many features (such as biocompatibility, biodegradability, physical, chemical and mechanical properties, growth factors binding capabilities and biological cues) have been utilized for engineering 3D scaffolds providing ECM microenvironment that enable endogenous or transplanted ‘exogenous' cells to growth and differentiate. Novel therapies to ameliorate neurodegeneration and brain injuries are being developed from innovative biomaterials; in particular new advanced in material design, such as controlled nanofiber diameter, alignment and interfiber distances are facilitating neural attachment and neurite growth targeting tissue repair (Lee et al., 2013, Lee et al., 2015). Progress in neural monitoring through versatile and biocompatible materials facilitate to understand neural processes at a more elemental level as well as monitor repair *in vivo*. The importance of modifying scaffolds to better mimic the natural developmental microenvironment of neurons bases on success in rebuilding neural network (Baranes et al., 2012). Hence, manipulation of topography, stiffness, electrical properties and integration of biological and chemical signals enhance growth, adhesion, proliferation and differentiation monitoring neural dynamics. Materials properties and characteristics are compared for impact on neural development (**Table 1**). Among these materials, several candidates have been studied for neural tissue engineering including

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hydrogels, electrospun nanofibers and self-assembling peptides scaffolds. Each scaffold exhibits variations in morphology due to distinct manufacturing techniques, which depends on the application. A brief summary table of scaffold materials used for 3D NS/NPC culture is provided (**Table 2**). The interested reader is also directed to the following reviews and papers relating to the deployment of biomaterials of neural repair (Bruggeman et al., 2018, Nisbet et al., 2008, Wang et al., 2012a, Horne et al., 2010, Wang et al., 2014)

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Future directions and conclusions

More direct collaboration is called for between the fields of biomaterial engineering and neurodevelopmental biology. The study of neural development is limited both *in vitro* and *in vivo*. *In vitro*, the 2D and other inadequately biomimetic culture systems has led to uncharacteristic cell behaviour, and unrepresentative data. This is established and accepted, with bioengineered 3D culture systems agreed to produce more *in vivo* like cell behaviour. However, there is unrealised potential for biomaterials to also improve ongoing *in vivo* research as well.

In vivo studies ensure natural cell behaviours, and therefore provide more accurate observations, but are subject to their own issues with costs, ethics, and variability. In addition, *in vivo* studies also carry a greater challenge in accessing results simply because it is difficult to visualise cellular processes without perturbing the system (Dhar et al., 2018). This has motivated advancements in microscopy to allow better and deeper visualisation (Wang et al., 2019), but the inability to directly and constantly observe *in vivo* studies at a cellular level still limits the results they can provide. It is also very difficult to investigate non-standard conditions. Investigating any parameter *in vivo* requires a means of naturally inducing those parameter changes, which can be a very complex process (Campos et al., 2014).

Collaboration and feedback between engineering, primarily focused on biomaterials as therapeutics, and the biological study focused on understanding neural development, would be mutually beneficial. As biomaterials are more and more able to match natural ECM and induce *in vivo*-like cell behaviour/s, they provide an '*ex vivo*' alternative to *in vivo* studies, enable more detailed observation, greater control of environmental conditions, and a wider more easily achieved range of conditions. It is easier to observe biomaterials than deep brain tissue, easier to specifically control culture conditions compared to brains across different animals, and easier to synthetically vary test conditions with biomaterials than to find a natural pathway to induce the desired condition. Meanwhile, the design of bioengineered materials is based on the initial biological investigation of cell behaviour, and could only be made easier and more accurate if that investigation included the materials and material components being used. A better understanding of how neural cells interact with biomaterials at the most basic level would inform future material design towards improved therapeutic benefits.

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Biomaterials mimicking the natural brain ECM aren't perfect, but results from their use in regenerative medicine indicate that they are often advantageous, and combined with their synthetic control and high throughput synthesis, they could offer an alternative approach to neural development research questions that have previously been restricted to *in vivo* experimentation. This shift, from a pure but limited sample to a potentially imperfect but much larger data set, is a common feature of many fields in the age of big data. Analysis of google search trends inform research into previously unknown drug side effects (White et al., 2016), analysis of personal fitness tracker data has provided insights into cardiovascular disease (Lim et al., 2018), and 3D printing of models from a large library of CT scans has replaced the much slower process of modelling from limited bone samples (Banerjee et al., 2014). The use of brain mimicking-biomaterials could also be seen as a shift from top down (investigating deeper into existing brain structures) to bottom up (creating more and more complex synthetic brain structure mimics), and again this is in line with other fields that have seen this shift caused by nanotechnology and additive manufacturing.

One of the reasons to explore neural development is to understand how things work when they are working, so as to be able to help fix them when they don't. Yet the investigation of neural development and neural therapeutics seem to have diverged when it comes to the use of advanced biomaterials. Biomaterials should be adopted more within the study of neural development, improve the state of both that field and therapeutic biomaterials.

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Declaration of interests

The authors declare no conflicts of interest.

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Figure Legends

Figure 1. Cellular organization and differentiation in the developing neocortex. *Refer to the text for a description of processes of the neurogenesis. Figure reproduced with permission from (Arai and Taverna, 2017). Copyright © 2017 Arai and Taverna.*

Figure 2. Cellular organization within the human SVZ. *The human SVZ consists of four distinct layers. Refer to the text for an explanation of the zones. Figure reproduced with permission from (Arias-Carrion, 2008). Copyright © 2008 Arias-Carrión.*

Figure 3. The extracellular matrix composition. *The brain extracellular matrix consists in three major regions: the basal lamina, the perineural net and the neural interstitial matrix. Image reproduced with permission from (Kim et al., 2018). Copyright © 2018 Kim, Meade, Chen Feng, Rayyan, Hess-Dunning and Ereifej.*

Figure 4. Physical structure regulates cell behavior. *(1) Different fibers diameters impact on NSCs differentiation, with larger fibers favoring TUJ+ neuronal progenitors and small scaffold fibres preferencing oligodendrocyte progenitors. Image reproduced with permission from (Christopherson et al., 2009). Copyright © 2008 Elsevier Ltd. All rights reserved. (2) SEM images confirm that cells on small fibers (pane d-f) present a stretched morphology similar to oligodendrocytes while cells on larger fibers (panels i-j; g-h) show a similar morphology to neural progenitors, extending neurites preferentially along the scaffold fiber axis. Image reproduced with permission from (Christopherson et al., 2009). Copyright © 2008 Elsevier Ltd. All rights reserved. (3) Surface topography controls cell shape: neurospheres plated on small PCL fiber mats coated with PDL (panels B and C show migratory morphology (green arrow in panel C indicates extended processes) while neurospheres plated on larger PCL fiber mats, also coated with PDL, show less interaction (panels F and G) and spherical morphology (light blue arrow panel G). Small PCL fiber mats coated with laminin show bipolar morphology (panel E, white arrows) and big PCL fiber mats induce migration of precursors out of neurospheres along the fibers. Image reproduced with permission from (Czeisler et al., 2016). Copyright © 2016 Wiley Periodicals, Inc.*

Figure 5. SAPs as relevant biomimetic scaffolds. *(1) A. Chemical structure of Fmoc-DDIKVAV. B-E. Schematic of Fmoc-assembly process: π-β structure assembly resulting in nanofibers with the Fmoc groups in the core and the peptide sequence exposed to the outised. Figure reproduced with permission from (Somaa et al., 2017). Copyright © 2017 The Authors. (2) The fibrous network can shear-encapsulate proteins such as BDNF to provide sustained delivery and at the same time*

structural and chemical support to cells (Nisbet et al., 2018). (3) Treatment of primary cortical neurons with soluble BDNF and conditioned media from SAP-BDNF hydrogels shows elevated metabolic activity. Image reproduced with permission from (Nisbet et al., 2018). Copyright © 2018 WILEY□VCH Verlag GmbH & Co. KGaA, Weinheim. (4) BDNF functionalized SAPs can influence human NSCs implanted in vivo, resulting in increased survival and differentiation. Image reproduced with permission from (Nisbet et al., 2018). Copyright © 2018 WILEY□VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 6. A brief timeline of key milestone in neural stem cells and biomaterials advances. *These contributions helped to set base for nowadays advances in neural tissue engineering. NSCs discovery and isolation and the ability to engineer biocompatible materials suitable to the CNS have permitted to merge neural stem cell therapies with 3D biomaterials encouraging repair and reconstruction within the CNS.*

Figure 7. Nanotechnology approaches to direct stem cell-based neural regeneration. *Nano-scaffolds offer the great promise to generate tools suitable for neural applications. Soluble factors play an important role in directing the stem cells fate: to overcome challenges, advanced nanoparticle systems have been used to direct efficient delivery to control differentiation. Patterned surfaces have been utilized to guide neural differentiation and polarization. Image adapted from with permission from (Shah et al., 2016). Copyright © 2016, American Chemical Society.*

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Table 1. Beneficial properties of Biomaterials

Material property	
ECM topography and rigidity[a]	Mimicking the native neural physical environment resulting in increment of neural adhesion, neurite growth and guided direction.
Stiffness[b]	Differences in stiffness influence neurite length and/or improve network connectivity and directs stem cell differentiation.
Electrical[c]	Electrical stimuli increase neurite length and polarization and migration of neurons. Improve neural differentiation.
Presentation of biological and chemical clues[d]	Presentation of specific small molecules/pepties/proteins support survival, neural growth, proliferation, differentiation.

[a](Chua et al., 2014) [b](Zhang et al., 2014) [c](Koppes et al., 2016) [d](Yang et al., 2015)

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Table 2: Biomaterials and their application in supporting neural cells *in vitro* & *in vivo*

Scaffold	Method	Cell type	Outcome	Reference
Natural biomaterials				
Type-I Collagen	Hydrogel	Embryonic rat neural stem and progenitor cells	Functional synapse and neuronal network formation in a 3D matrix	(Ma et al., 2004)
Type-I Collagen/hyaluronic matrix	Hydrogel	Embryonic and adult mouse neural stem cells	Survival, proliferation and differentiation of NSCs and NPCs compared to 2D culture	(Brannvall et al., 2007)
Alginate	Hydrogel	Adult rat neural stem cells	First demonstration of the influence of modulus on NSC differentiation in 3D scaffold	(Banerjee et al., 2009)
Chitosan	Hydrogel	Embryonic rat neural stem cells	Demonstration of the role of topology in regulating differentiation and proliferation of NSCs in chitosan hydrogels	(Wang et al., 2010)
Hyaluronic acid	Hydrogel	Ventral midbrain-derived mouse neural stem cells	Different mechanical properties influence on the differentiation of NPCs into astrocytes or neurons	(Seidlits et al., 2010)
Synthetic biomaterials				
Mixture of poly(ethylene glycol) (PEG) and poly(L-lysine) (PLL)	Hydrogel	Mouse postnatal isolated neural stem cells	The mechanical modulus of cross-linked hydrogels (PEG/PLL) impacts NSC migration and differentiation	(Hynes et al., 2009)

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IKVAV-RADA16 Self-assembling peptide	Hydrogel	Primary mouse neural stem cells	Self-assembling peptide 3D culture for neural tissue applications	(Zhang et al., 2010)
Nanofiborus poly(L-lactic acid) (PLLA)	Electrospinning	Immortalized mouse neural stem cell line (C17.2)	Nanofibrous scaffold support NSC differentiation, neurites out-growth and NSC adhesion	(Yang et al., 2012)
Poly(ε-caprolactone) (PCL)	Electrospinning	Mouse cortical NSC/progenitors	Electrospun fibres influence NSC/progenitor proliferation, differentiation and neurite growth.	(Wang et al., 2012a)
Fmoc-self-assembling peptides (Fmoc-SAPs)	Hydrogel	Mouse cortical NPCs	SAPs as a tool for cell transplantation	(Rodriguez et al., 2014)
IKVAV-RADA16 Self-assembling peptide	Hydrogel	Rat neural stem cells	IKVAV-RADA16 support encapsulated NSCs and reduced the formation of glia astrocytes	(Cheng et al., 2013)

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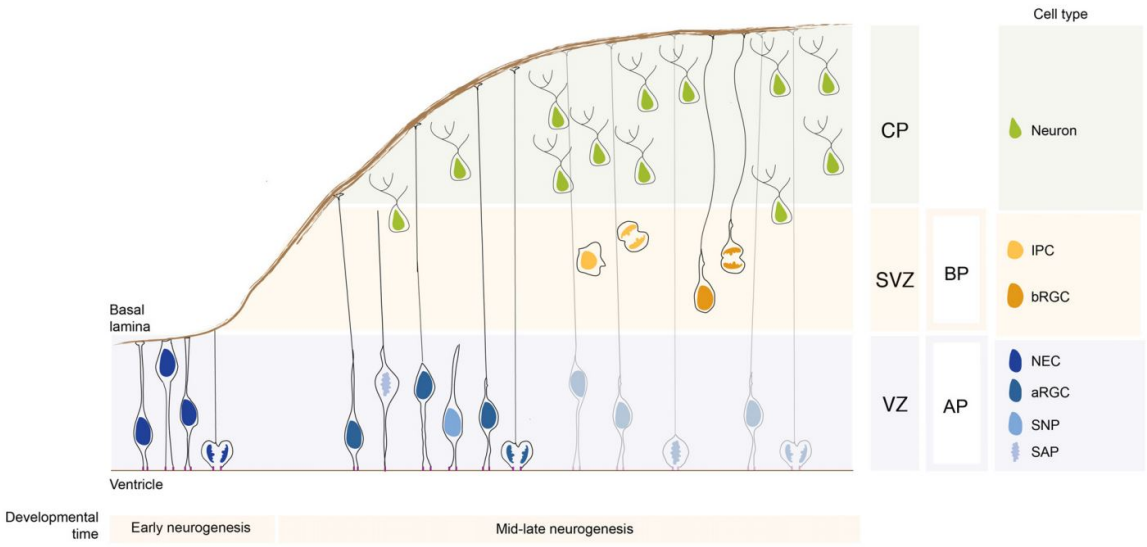

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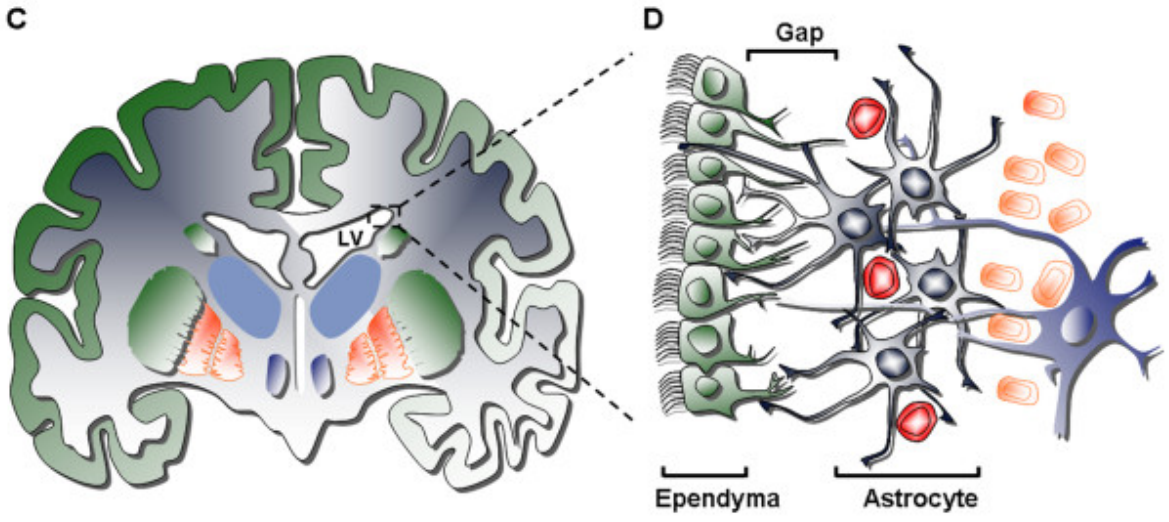

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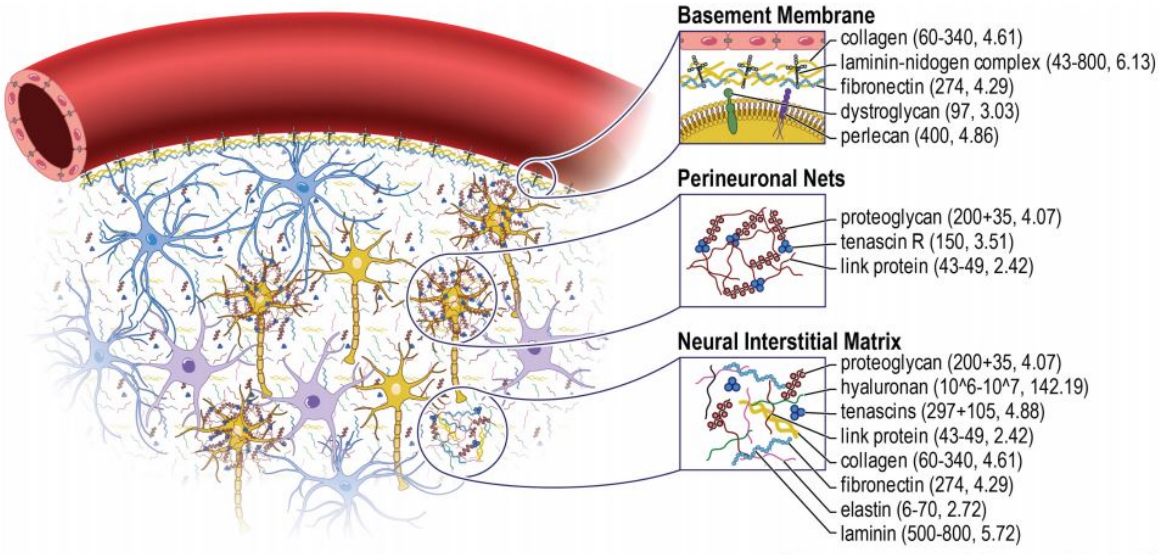

Figure courtesy of Cleveland FES Center

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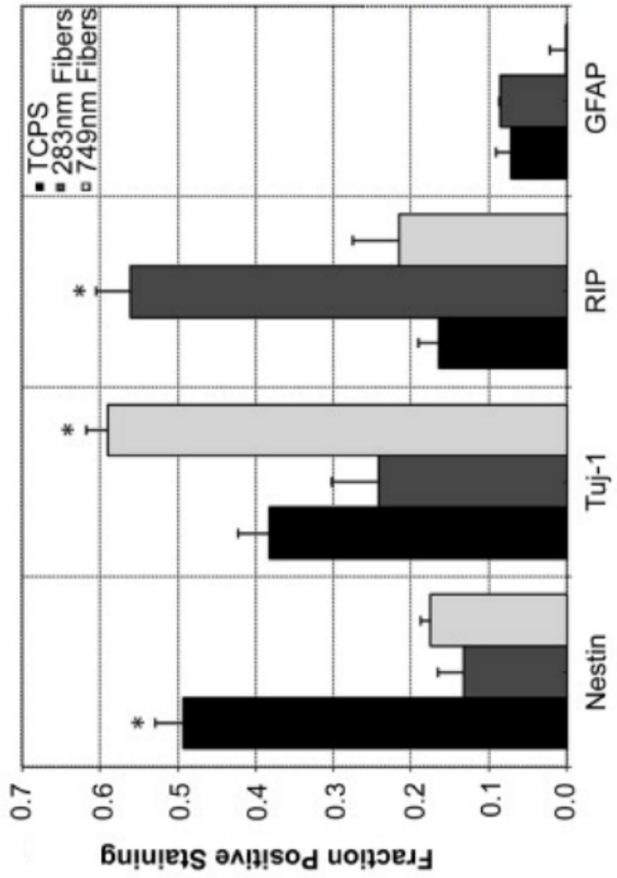
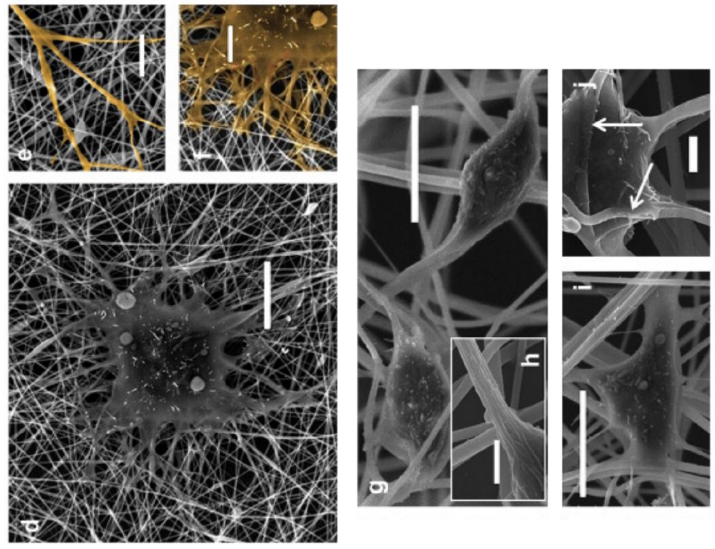
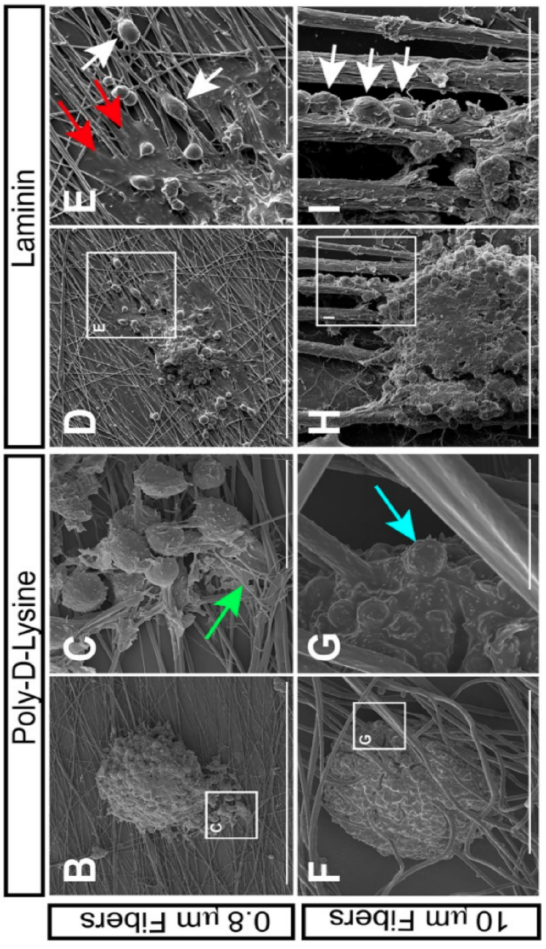
1.

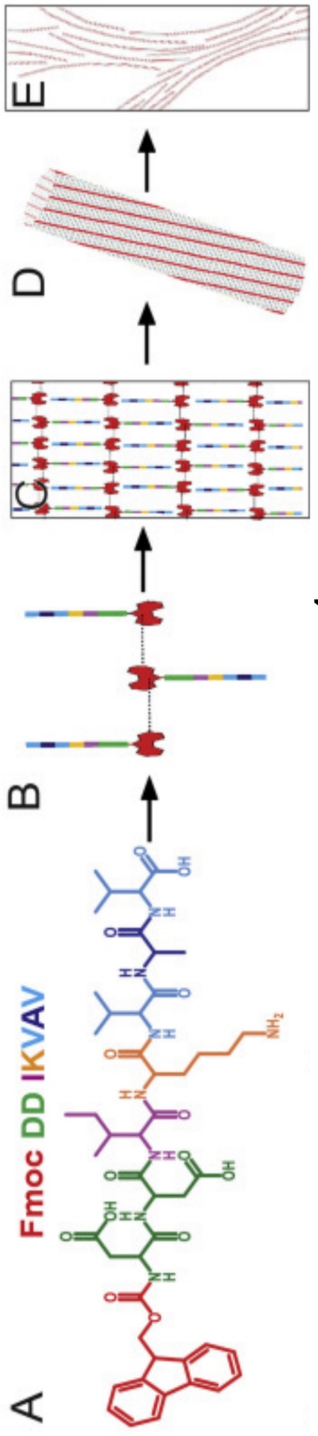

Fraction Positive Staining
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
TCPS
283nm Fibers
749nm Fibers
*
*
*
Nestin
Tuj-1
RIP
GFAP

2.

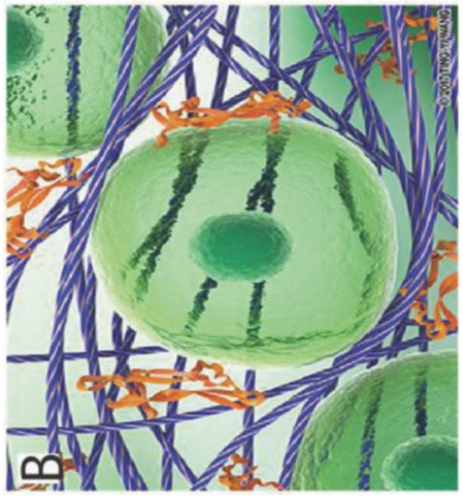

d
e
f
g
h
i
i

3.

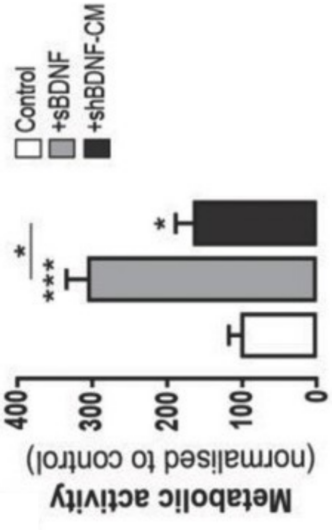

Poly-D-Lysine
Laminin
0.8 µm Fibers
10 µm Fibers
B
C
D
E
F
G
H
I


A
Fmoc DD IKVAV
B
C
D
E

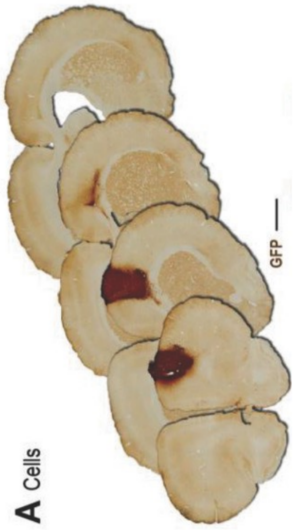
1.


B

2.

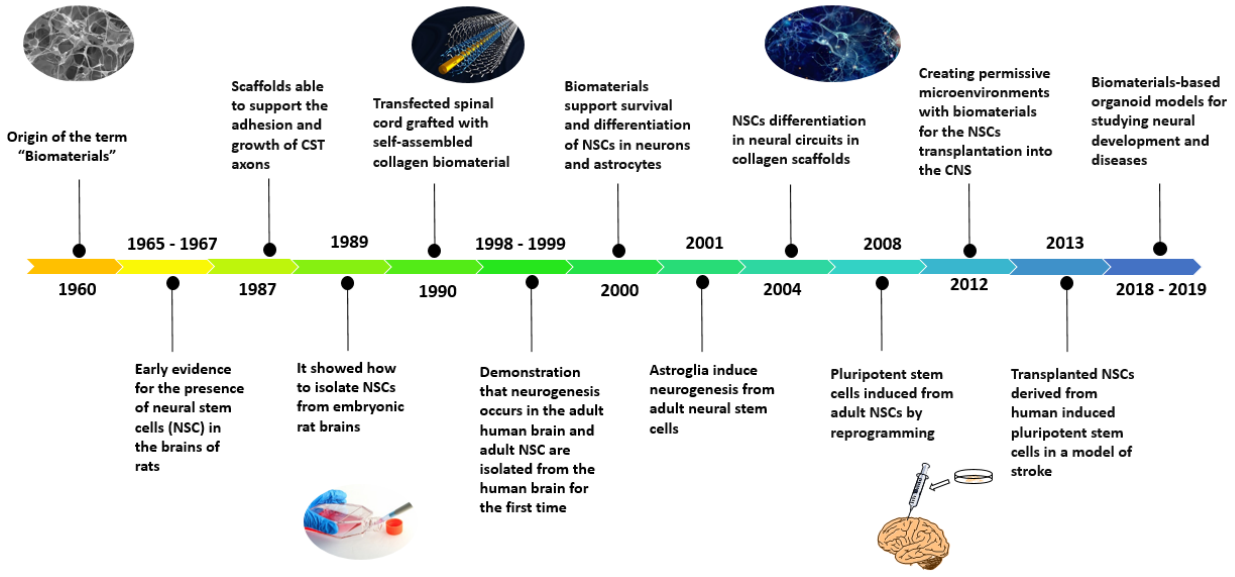

Control
+sBDNF
+shBDNF-CM
Metabolic activity
(normalised to control)
400
300
200
100
0
*
***
*

3.


A Cells
GFP

D Cells+SAP-shBDNF

4.



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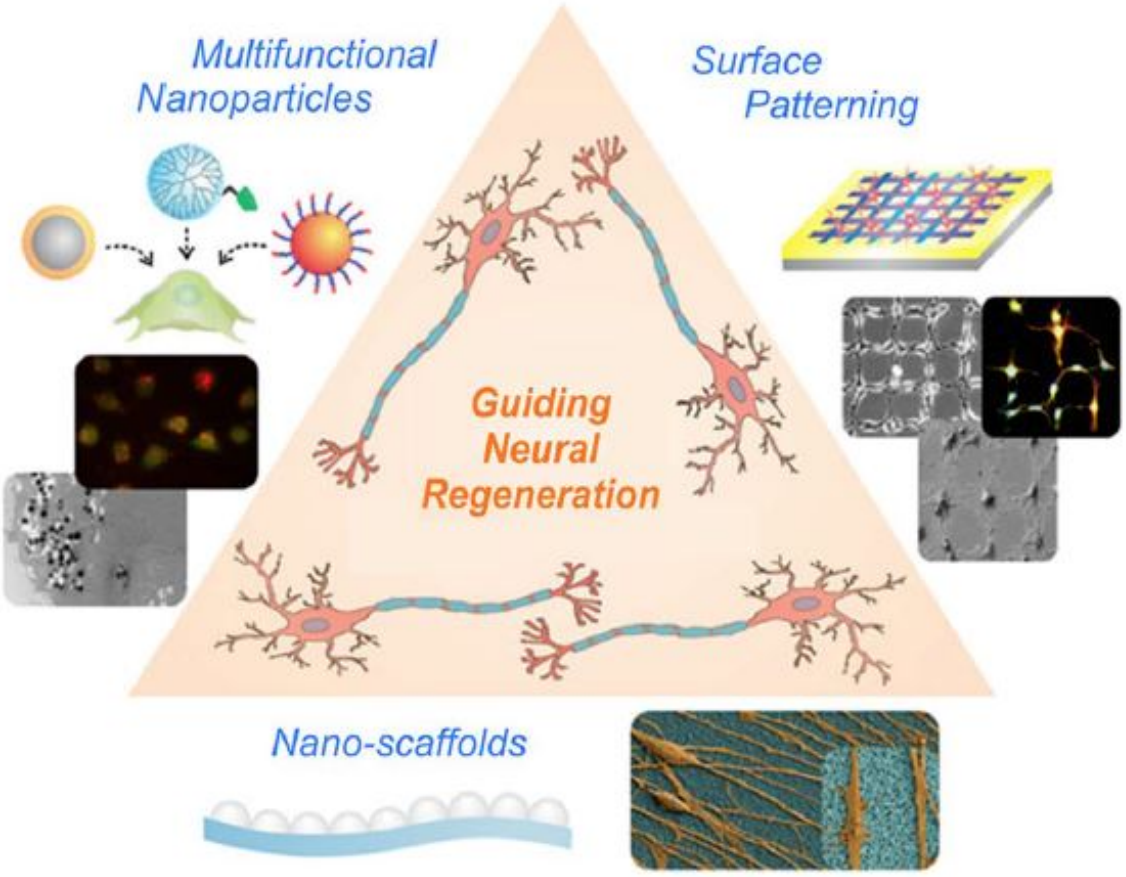
Multifunctional
Nanoparticles
Surface
Patterning
Guiding
Neural
Regeneration
Nano-scaffolds

Journ