ARTICLE IN PRESS

Biotechnology Advances xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Neural tissue engineering with structured hydrogels in CNS models and therapies

Julian George[1], Chia-Chen Hsu[1], Linh Thuy Ba Nguyen, Hua Ye*, Zhanfeng Cui*

Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, Oxford, United Kingdom

A R T I C L E I N F O

Keywords:
Extracellular matrix
Neuron
Structured Hydrogel
Microgel
Granular hydrogel
Tissue Engineering
Regenerative Medicine

A B S T R A C T

The development of techniques to create and use multiphase microstructured hydrogels (granular hydrogels or microgels) has enabled the generation of cultures with more biologically relevant architecture and use of structured hydrogels is especially pertinent to the development of new types of central nervous system (CNS) culture models and therapies. We review material choice and the customisation of hydrogel structure, as well as the use of hydrogels in developmental models. Combining the use of structured hydrogel techniques with developmentally relevant tissue culture approaches will enable the generation of more relevant models and treatments to repair damaged CNS tissue architecture.

1. Introduction

Neural damage remains one of the most untreatable causes of long-term disability (DiLuca and Olesen, 2014), and it is hoped that advances in regenerative medicine such as neuron replacement therapy will lead to new types of effective treatment (Grade and Götz, 2017). A major hurdle to the development of regenerative medicine approaches is the inability to adequately reverse and restore loss of the micropatterned extracellular microenvironment that contains the developmental context for tissue homeostasis and long term function (Han et al., 2014; Sachs et al., 2017; Williams and Lavik, 2009). This developmental context is destroyed at the point of damage, and in the case of traumatic injury, replaced with scar tissues (Fitch and Silver, 2008) that acts as a barrier to regeneration and perpetuates local dysfunction (Karve et al., 2016).

Slowly progressing conditions such as Alzheimer's disease also lead to disorganisation of the tissue structure via a number of mechanisms. These include the accumulation of extracellular amyloid plaques, the overexpression of laminin and the production of intracellular aggregates of hyperphosphorylated tau protein that modulate cell function and lead to further modulation of the surrounding tissue architecture (Palu and Liesi, 2002). Whilst it has been shown that it may be possible to regenerate large sections of lost CNS tissue in animal models, such as the Axolotl model (Amamoto et al., 2016), loss of the developmental context can result in highly disorganised regrowth, indicating loss of function. To overcome permanent loss of developmental context, it should be possible to prime tissue regeneration through incorporation of engineered developmentally relevant microenvironments that bootstrap the tissue regeneration process (Sachs et al., 2017). To achieve this, control must be gained over the generation and delivery of patterned microenvironments into tissues targeted for regeneration.

Hydrogels are commonly chosen and adapted for use as support matrices for neural cultures (Aurand et al., 2012) (Fig. 1). Many different techniques have been used to add microstructure to hydrogels for different applications (De France et al., 2018; Ma et al., 2016; Sano et al., 2018; Zhao et al., 2017b). More commonly used approaches include the addition of microscale particles with anisotropic shape that can be aligned with physical force (Marelli et al., 2015), and physical confinement of the hydrogel to create aligned fibrils (S. H. Kim et al., 2017a; Mredha et al., 2018). Magnetic fields have also been used to directly modulate the alignment of magnetic particles within a hydrogel (Antman-Passig and Shefi, 2016; Kim et al., 2016) or indirectly align hydrogel fibrils (Ceballos et al., 1999). It is also possible to use fabrication techniques to produce arbitrarily complex microscale patterns within hydrogels, such as the use of photolithography to achieve aligned three dimensional (3D) molecular printing (Aguilar et al., 2018).

In this review we provide a broad overview of the use of hydrogels in CNS regenerative medicine applications, establishing levels of complexity ranging from material choice to the fabrication of dynamic perfused systems that use structured hydrogels. We also draw on recent

* Corresponding authors.
E-mail addresses: hua.ye@eng.ox.ac.uk (H. Ye), zhanfeng.cui@eng.ox.ac.uk (Z. Cui).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biotechadv.2019.03.009
Received 26 October 2018; Received in revised form 25 February 2019; Accepted 11 March 2019
0734-9750/ © 2019 Elsevier Inc. All rights reserved.

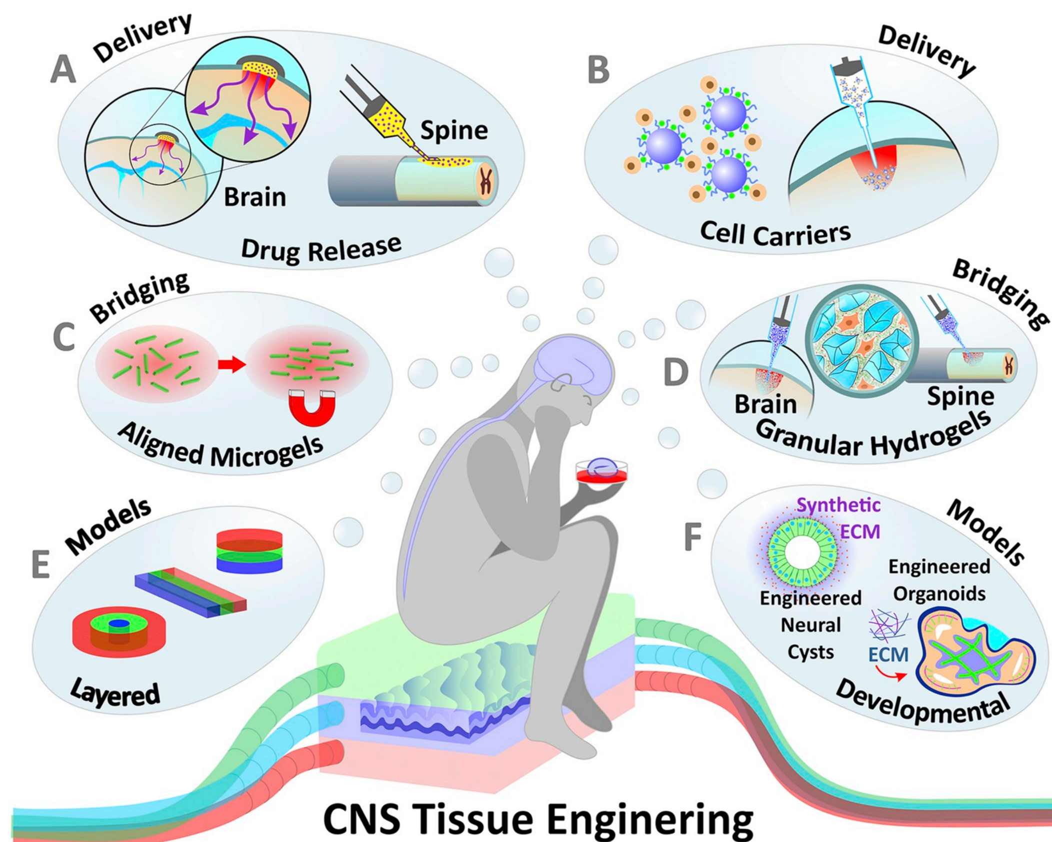

Fig. 1. Structured Hydrogels in CNS models and therapy. (A) Hydrogels have been proposed for use in controlled release of soluble factors for drug delivery applications (Tam et al., 2014), (B) as carriers that support cell transplantation (Skop et al., 2016), (C) as aligned hydrogels that guide axon growth and neurite orientation (Omidinia-Anarkoli et al., 2017), (D) and as bridging scaffolds composed of microgels and granular gels that facilitate neuronal growth across damaged tissues (Chedly et al., 2017; Lozano et al., 2016; Nih et al., 2017). (E) Layered hydrogel architectures have been used to spatially compartmentalise neurons to replicate aspects of in-vivo tissue structure (Kunze et al., 2011; Tang-Schomer et al., 2014) (F) and hydrogels have also been instrumental in the generation of neuroepithelial cysts and CNS organoids used as in-vitro developmental models (Lancaster et al., 2017; Ranga et al., 2016). ECM: extracellular matrix.

insights gained from the generation of neural organoid models (Lancaster et al., 2017, 2013), highlighting how hydrogel use plays a key role in the establishment of developmental context within these systems. Finally, we highlight the challenges and opportunities that use of structured hydrogels can bring to future studies.

2. A hierarchy of hydrogel structure

Tissues are dynamic systems that build in complexity during development until a state of homeostasis is reached in adulthood. Cells, as the principal agents of tissue function, continuously adjust internal state in response to environmental factors. The extracellular environment, as a secondary agent, is also dynamically changing and encompasses all external stimuli, including the extracellular matrix (ECM), soluble factors and cell-to-cell interaction. A third agent, albeit a non-physical abstraction, is the hierarchically structured arrangement of cells and ECM within the extracellular environment. This structured arrangement increases in complexity throughout development and whilst control can be gained over cell state through use of differentiation protocols, and over the extracellular environment through the selection of materials, controlling the 3D structural arrangement of cells and replicating natural tissue physiology in-vitro is significantly challenging. The structured environment can be broken into a hierarchy of levels based on scale and information content (Fig. 2), and we use this format to explore the role of the structured environment in relation to CNS culture and therapy.

Order	Structure	Examples
1st	Chemical interaction	Absorption Diffusion Biological binding Cross-linking, ...
2nd	Hydrogel bulk structure	Pore size and porosity Stiffness and elasticity Swelling, Degradation ...
3rd	Multi-phase structure	Spheres Granules Fibres ...
4th	Single axis alignment	Shear alignment Magnetic alignment Self-alignment ...
5th	Multi-axis patterning	Moulding 3D printing Photolithography ...
6th	Extrinsic factors	Micro-fluidic systems - micro-bioreactors - lab-on-chip ...

Fig. 2. Hierarchical hydrogel structure. Hydrogel structure can be ordered by increasing scale and information content. Following material choice, hydrogels can be hierarchically structured on multiple levels of increasing scale (1st to 3rd order) and information content (4th to 6th order). The final level (6th order) represents a spatially and temporally well-controlled environment, capable of replicating patterning cues established during the neural development.

2.1. Hydrogel chemical interaction (1st order)

At the lowest level of organisation (1st order), biophysical interactions within a hydrogel occur at the molecular scale, with solutes dissolved in the aqueous phase interacting with hydrated polymer chains. Diffusion into and out of cellular systems is a necessary requirement to support all aspects of cellular function. The rate of diffusion depends on the number and type of physical interactions that solutes encounter when passing through the hydrogel (Amsden, 1998). Denser hydrogels and charged hydrogels can support slower rates of diffusion by increasing the interaction of the matrix with solutes. This acts to enable the establishment of gradients of factors and entrap expressed factors close to cells (Lühmann and Hall, 2009). Hydrogel chemistry can also be modulated to support the controlled release of factors for use in drug delivery applications (Jiang et al., 2014; Li and Mooney, 2016). Using methods of chemical conjugation, physical incorporation and drug impregnated polymeric microspheres, hydrogels have been used to control the release of nerve growth factor (NGF) (Dodla and Bellamkonda, 2008; Kuo and Chang, 2013), brain-derived neurotrophic factor (BDNF) (Cook et al., 2017; Park et al., 2010), glial cell-derived neurotrophic factor (GDNF) (Ansorena et al., 2013; Fon et al., 2014; Moriarty et al., 2017), neurotrophin-3 (NT-3) (Stanwick et al., 2012), insulin-like growth factor 1 (IGF-1) (Lee et al., 2014), fibroblast growth factor (FGF) (Chen et al., 2015; Das et al., 2017; Freudenberg et al., 2009), epidermal growth factor (EGF) (Egawa et al., 2011), as well as vascular endothelial growth factor (VEGF) (Gnavi et al., 2017; Lee et al., 2010).

2.1.1. Choice of hydrogel material

Many different hydrogel materials have been investigated for use in the development of CNS models and applications (Fig. 3, Table 1). Cells of the CNS are attachment dependent and culture matrices need to provide attachment sites for cell receptors, for example, through inclusion of integrin (Yang et al., 2015) or cadherin (Vega et al., 2016) binding peptides. Cells also bind to each other or indirectly bind to ECM that has been expressed or otherwise deposited within the hydrogel. The amount of ECM and other biological factors that become absorbed or entrapped within the hydrogel matrix affects the establishment of the cell niche (Scadden, 2014). This ECM response is directly modulated by hydrogel chemistry and indirectly modulated by the rate and size of products that can diffuse through the matrix. Cell morphology, local exploration and migration are modulated by many hydrogel factors, including scaffold stiffness, porosity and the availability of binding sites. Furthermore, the establishment of gradients or discontinuities of mechanical or biological properties within a hydrogel can be used to pattern cell behaviour (Turunen et al., 2013). Cost, availability, biological relevance and variability also determine material choice, with biologically extracted composite products having greater variability in comparrison to synthetic products.

Biological components form a major structural component of the CNS extracellular environment. Whilst it is not currently possible or cost effective to synthesise many of these polymers, an alternative approach is to isolate and purify these polymers from natural sources (Crapo et al., 2012). Extracts from decellularized brain matrix are rich in glycosaminoglycans (GAGs) and also contain collagen (I, III, IV, V, and VI), perlecan, fibronectin and laminin, whereas decellularized spinal cord has been found to contain fibronectin, collagen IV, laminin, and GAGs (DeQuach et al., 2011; Xu et al., 2016). These extracts can be solubilised and neutralised by temperature- or pH-controlled methods and then gelled through reformation of the intramolecular bonds (Saldin et al., 2017). In this reconstituted form they have been shown to support cell growth, differentiation and neurite extension (Bible et al., 2012; DeQuach et al., 2011; Medberry et al., 2013; Xu et al., 2016). Alternatively, basement membrane extract expressed by cultured mouse sarcoma cells (commercially available as Matrigel™ or ECM gel) is commonly used to promote cell attachment. Composed primarily of

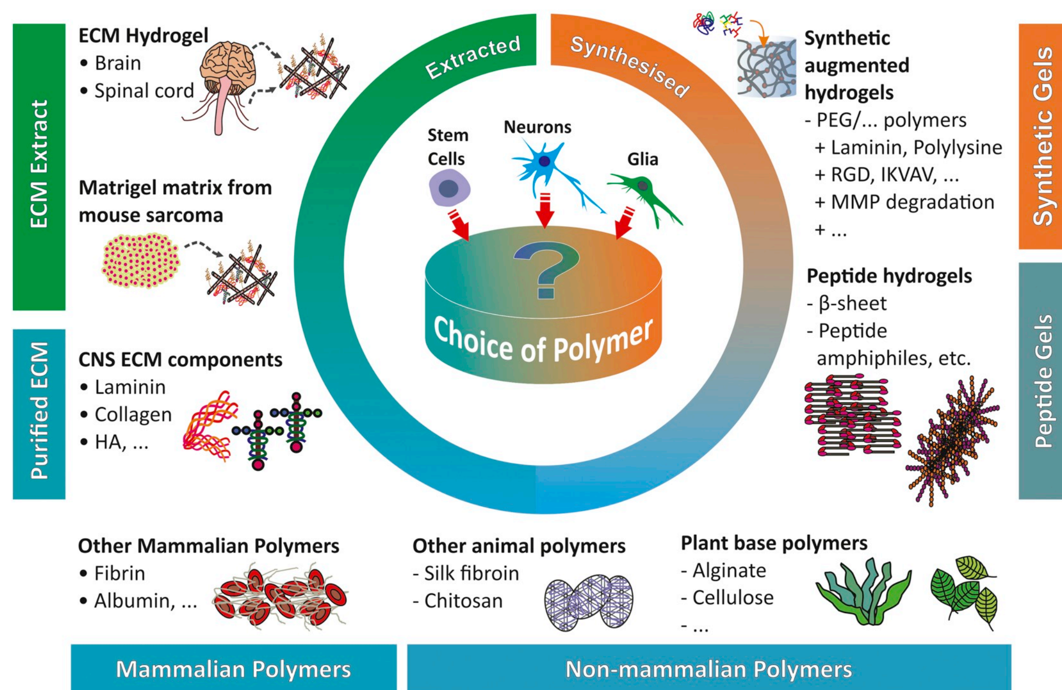

Fig. 3. Different sources of polymer used in hydrogel preparations for CNS applications. Hydrogel choice is motivated by many factors including cost, availability, variability and biological relevance. Whilst hydrogels composed of proteins and proteoglycans extracted from CNS tissue or from other sources such as mouse sarcoma basement membrane extract (Matrigel) can be readily bound to by cell membrane integrins, they can also be degraded by cells and may need additional crosslinking to support longer term used. The heterogeneity of extracted products is also an issue, and this can be overcome by making use of synthetic polymers together with defined cross-linking strategies. Hydrogels extracted from non-mammalian sources will not bind integrins, and the addition of a biological binding agent such as Laminin or an integrin binding peptide sequence may be necessary. ECM: extracellular matrix; HA: hyaluronic acid; PEG: poly(ethylene glycol); RGD: Arg-Gly-Asp; IKVAV: Ile-Lys-Val-Ala-Val; MMP: matrix metalloproteinases.

Table 1
Characteristics of common hydrogel materials for CNS and their advancements.

Material	Gelation mechanism	Key characteristics	Summary of advances made	Significant biological responses	Ref
Decellularised ECM hydrogel	pH, temperature	✓ Containing cell binding ligands. ✓ Retaining trophic proteins and growth factors. × Not well characterised. × Limited availability of clinically relevant materials. × Batch to batch variation.	First decellularised brain ECM.	Higher dendritic processes and increased maturity.	(DeQuach et al., 2011)
ECM gel	Temperature	✓ Containing cell binding ligands. × Not well characterised. × Batch to batch variation. × Xeno- and tumorigenic origin. × Weak mechanical properties and fast degradation.	Matrigel droplets were used to develop human pluripotent stem cell-derived cerebral organoids.	Cerebral organoids can recapitulate developing human brain organisation at the early stage.	(Lancaster et al., 2013)
Collagen	pH, temperature, chemical crosslinking	✓ Containing cell binding ligands. ✓ Facile gelation under physiological conditions. × Not primary CNS ECM protein.	Collagen hydrogels with uniaxial alignment tethered by physical cues and cells.	Aligned collagen gels oriented neurons in-vitro and could improve neuronal regeneration.	(Abu-Rub et al., 2011; Lanfer et al., 2008; Rose et al., 2017; Phillips et al., 2005; Kim et al., 2017b; Alexander et al., 2006)
Hyaluronic acid (HA)	Thermal/ionic/ chemical crosslinking	✓ CNS structural backbone. ✓ Binding to other ECM components, such as proteoglycans. ✓ Regulating inflammation, glial scar formation, and angiogenesis. ✓ Similar mechanical properties to adult CNS tissues. × Without inherent cell adhesiveness.	HA hydrogels were tailored with different bio-functionalisation and mechanical properties.	Cell adhesion was improved and different mechanical properties affected cell differentiation.	(Segura et al., 2005; Seidlits et al., 2010)
Fibrin	Chemical crosslinking	✓ Containing ligands binding to integrins, growth factors, and ECM proteins (e.g. fibronectins). ✓ Involved in native nerve repair processes and regulating microglia activation. ✓ Good mechanical properties and porosity. × The autofluorescence of the high concentration of fibrinogen/fibrin might hinder fluorometric staining methods.	Fibrin hydrogels were functionalised as growth factor release systems by covalently crosslinking with a synthetic peptide, which can bind to heparin.	Fibrin hydrogels NT-3 delivery system increased DRG neurite extension and enhanced neuronal fibre sprouting in an in-vivo SCI rat model.	(Taylor et al., 2004; Taylor et al., 2006)
Silk fibroin	pH, temperature, vortex shearing, ultra-sonication, chemical crosslinking	✓ Slow degradation (months) and slow release of loaded bioactive factors ✓ The bulk mechanical properties fall in the range for soft tissues. ✓ Low immunogenicity. × Without inherent cell adhesiveness and ligands binding to growth factors and ECM proteins.	Silk hydrogels which degrade slowly might allow sufficient time for in-vivo repair and benefit slow release of bioactive factors.	Silk hydrogels functionalised with fibronectin and NT-3 increased neurite outgrowth and the hydrogels with modulate stiffness promoted greater neurite outgrowth.	(Hopkins et al., 2013)
Chitosan	Ionic/chemical crosslinking	✓ Low cost. ✓ Low or non-immunogenicity. ✓ Acquiring similar linear structures as HA, the major CNS ECM. ✓ The positive charge can enhance cell attachment. × Without inherent cell adhesiveness and ligands binding to growth factors and ECM proteins.	Photocrosslinked methacrylamide-chitosan hydrogels were synthesised with cell adhesive peptides or immobilised with bioactive factors using recombinant protein technology.	Cells acquired higher lineage specific differentiation. The in-vivo hydrogel implantation showed mild immune responses in rats.	(Leipzig et al., 2011; Li et al., 2014)
Alginate	Ionic/chemical crosslinking	✓ Low cost. ✓ Ease of cell encapsulation. ✓ Low or non-immunogenicity.	Functionalisation of alginate hydrogels with immobilized cell-adhesive peptides.	Functionalisation promoted cell adhesion and neurite outgrowth.	(Dhoot et al., 2004; Sandvig et al., 2015; Dalheim et al., 2016)

(*continued on next page*)

Table 1 (*continued*)

Material	Gelation mechanism	Key characteristics	Summary of advances made	Significant biological responses	Ref
		× Without inherent cell adhesiveness and ligands binding to growth factors and ECM proteins.			
Cellulose/cellulose derivatives	Temperature, chemical crosslinking	✓ Gelation occurs under physiological conditions. ✓ Low cost. ✓ Low or non-immunogenicity. × Without inherent cell adhesiveness and ligands binding to growth factors and ECM proteins.	Cellulose hydrogels synthesised via nanocarbon hybridisation exhibited lower mechanical property, increased porosity, and decreased fibril alignment.	Growth cones in the nanofibrillary hydrogels exhibited narrow spreading of actin filaments, enabling neurite elongation.	(Kim et al., 2017a)
Gellan gum	Ionic/chemical crosslinking	✓ Low cost. ✓ High gelling efficiency at body temperature. ✓ Suitable rheological properties. ✓ Excellent optical clarity. × Without inherent cell adhesiveness and ligands binding to growth factors and ECM proteins.	Functionalisation of gellan gum (GG) hydrogels with immobilized cell-adhesive peptides.	Neural progenitors cultured on GG–GRGDS hydrogels acquired better cell adhesion and proliferation.	(Silva et al., 2012)
Agarose	Temperature	✓ Good mechanical properties. ✓ Low cost. ✓ Low or non-immunogenicity. × Without inherent cell adhesiveness and ligands binding to growth factors and ECM proteins. × No strong interaction with proteins and with relatively large pore sizes preclude long turn drug/protein release.	An agarose hydrogel modified with a cysteine compound containing a sulphydryl protecting group allows photo-patterning of biochemical cues in 3D hydrogel matrices.	Dorsal root ganglion cells grew into photopatterned GRGDS-oligopeptide-modified agarose channels but not in the surrounding areas.	(Luo and Shoichet, 2004)
Self-assembling peptides	pH, temperature, ionic crosslinking	✓ Facile modification through changes of the amino acid sequence, length of the peptide sequence, and addition of functional epitopes. ✓ More defined properties compared to natural hydrogels and less batch to batch variation. ✓ Gelation occurs under physiological conditions. ✓ Low or non-immunogenicity. × High cost. × Generally lack of long term stability.	The first rational design and detailed characterisation of self-assembling peptide hydrogels.	Peptide hydrogels supported neurite outgrowth and cell differentiation; however, it exhibited a lag of cellular responses compared to Matrigel.	(Banwell et al., 2009)
PEG	Chemical crosslinking	✓ Facile modulation of its structure, chemical and physical properties, and incorporation of biofunctionality. ✓ Facile and delicate control of the size and shape through electron beam lithography and photolithography. ✓ More defined properties compared to natural hydrogels and less batch to batch variation. ✓ Low or non-immunogenicity × Without inherent cell adhesiveness and ligands binding to growth factors and ECM proteins.	Defined PEG hydrogels were designed as modular matrices with different ECM molecules, various mechanical properties, and soluble factors for optimal neuroepithelial development.	An intermediate stiffness (E = 2 and 4 kPa) was required for optimal apical–basal polarity. A nondegradable matrix with laminin is required for proliferation, differentiation, and the establishment of neuroepithelial polarity. Neuroepithelial colonies in synthetic PEG matrices are more homogenous and polarised than in Matrigel.	(Ranga et al., 2016)

laminin, collagen IV, and enactin (Hughes et al., 2010), it is widely used to support neural cell culture and growth of neural organoid systems (Kothapalli and Kamm, 2013; Meinhardt et al., 2014; Tavakol et al., 2014); however high batch-to-batch variability and short degradation times as well as xeno- and tumorigenic origins are often cited as drawbacks (Ranga et al., 2016; Uemura et al., 2010; Hughes et al., 2010).

It is also possible to use purified extracts of the major CNS constituents. Commonly used purified extracts include hyaluronic acid (HA), laminin, collagen I and collagen IV. For example, HA can be extracted from animal tissues or produced xeno-free using bacterial fermentation (Liu et al., 2011). It has become one of the most commonly chosen materials for use in neural tissue engineering (Burdick and Prestwich, 2011; Khaing et al., 2011; Lin et al., 2009). Collagen has also been widely used to create hydrogels for neural culture (Ge et al., 2013; Gerardo-Nava et al., 2014; Herland et al., 2016; Swindle-Reilly et al., 2012), and laminin, which can enhance neural stem cell (NSC) expansion, migration and neuronal differentiation, as well as promote neurite outgrowth and neuronal network formation (reviewed in (Luckenbill-Edds, 1997)), is often incorporated as an adhesive protein in combination with other structural polymers (Deister et al., 2007; Lee et al., 2011; Swindle-Reilly et al., 2012; Yao et al., 2010).

Biological polymers used in CNS hydrogels can also be extracted and purified from sources with greater availability. Fibrin, derived from blood, has been used as a hydrogel for neural regeneration and delivery of neurotrophic factors (Lee et al., 2010; Navaei-Nigjeh et al., 2014; Scott et al., 2011a) and fibrin hydrogels have been shown to support neural cell growth, differentiation and neurite extension. However, they have also been found to block PNS axon remyelination and activate CNS microglia (Akassoglou et al., 2002; Ryu et al., 2009). Commonly investigated biopolymers used in neural culture include silk fibroin extracted from cocoons of silkworms (Hopkins et al., 2013; Sun et al., 2017), chitosan, a polycationic biopolymer derived from the exoskeleton of crustaceans (Crompton et al., 2007; Wei et al., 2016) with a linear structure similar to HA (Li et al., 2016; Tseng et al., 2015; Valmikinathan et al., 2012) and alginate, derived from brown algae (Ansorena et al., 2013; Onoe et al., 2016). Derivatives of cellulose plant extracts such as methylcellulose have also been used as hydrogels for neuronal culture (Kandalam et al., 2017; Stabenfeldt and LaPlaca, 2011; Tate et al., 2001).

Synthetic polymers offer a further class of materials for use in neural tissue engineering. Polypeptide hydrogels can be formed by peptide self-assembly and can be rationally designed to form supramolecular nanostructures, including cylinders, tubes and spheres (Matson and Stupp, 2012). β-sheet-based peptide hydrogels as well as α-helical peptide systems have been used to enhance NSC attachment, growth and differentiation (Cheng et al., 2013; Gelain et al., 2006; Mehrban et al., 2015). Furthermore, bioactive motifs can be designed into the peptide sequences to improve cell-matrix interactions. Peptide hydrogels that incorporate the IKVAV motif have been shown to promote cell attachment, neuronal differentiation and neurite outgrowth (Cheng et al., 2013; Gelain et al., 2006; Mehrban et al., 2015). Peptide hydrogels can also be tuned to match the mechanical properties of brain tissue (Cheng et al., 2013), for example, smooth, organised β-sheet structures have been found to generate stiffer hydrogels (Pashuck et al., 2010). Rationally designed peptide systems provide flexibility and facilitate the generation of tailored neural scaffolds. However, long-term stability remains challenging (Caliari and Burdick, 2016).

Many different non-biological synthetic polymers have also been investigated for use as CNS hydrogel scaffolds. Pluronic F127 undergoes the sol-gel transition at concentrations of 15-20% at room temperature (Strappe et al., 2005) and has been used to fabricate scaffolds that carry drugs, viral vectors and drug containing microspheres into the CNS (Sellers et al., 2014; Strappe et al., 2005; Wu et al., 2013). A commonly used synthetic hydrogel polymer is poly(ethylene glycol) (PEG), and PEG-based hydrogel constructs have been widely used to promote neuronal cell attachment, growth and differentiation, as well as serve as neural guidance conduits *in-vitro* (Aurand et al., 2014; Horn-Ranney et al., 2013; Lampe et al., 2010; Mahoney and Anseth, 2006; Namba et al., 2009; Royce Hynes et al., 2007; Tunesi et al., 2013; Zhou et al., 2012).

In summary, naturally derived polymers are commonly extracted and used to fabricate neural hydrogels, however, a common challenge with this approach is overcoming significant batch-to-batch variability over a wide range of biochemical and biophysical properties. Synthetic materials have well defined characteristics, however they often require additional functionalisation to support cell-matrix interactions. Recently, several studies have reported on the use of a modular approach to combine synthetic and purified natural materials to generate hydrogel scaffolds for cell studies (Ranga et al., 2016; Ranga et al., 2014; Nii et al., 2013; Mei et al., 2010), enabling the generation of well-characterised 3D matrix and providing independent control over a wide range of variables. This enables the tailoring of mechanical properties and proteolytic degradability, as well as the inclusion of signalling proteins related to cell-matrix interactions, cell-cell interactions, and soluble factors. These novel platforms provide flexibility and are suitable for use in high throughput investigations, enabling the study of cellular interactions with various factors simultaneously and in parallel.

2.1.2. Hydrogel crosslinking

The structural component of a hydrogel is composed of a dense collection of hydrated intermeshed polymer chains that form a swollen matrix within an aqueous medium. The properties of this matrix are extensively customisable through the use of chemical and physical crosslinking techniques (Fig. 4).

In general, there are four types of chemical crosslinking methods used to form hydrogels. Polymer chains can be conjugated through use of crosslinking molecules or can be directly bound to each other. The simplest form of crosslinking takes place between aldehyde and amino groups to form a Schiff base, where di-aldehyde crosslinkers (such as glyoxal and particularly glutaraldehyde) are used for hydrogelation (Wang et al., 2004) (Fig. 4A). Crosslinking molecules also present a facile method to bind biofunctional molecules into hydrogels. For example, a four arm PEG tetraazide crosslinker tethered with a laminin and a neurogenic factor, interferon-γ, has been used to fabricate a hydrogel for NSC encapsulation (Li et al., 2018). To generate polymer-polymer chains, polymers pre-functionalised with reactive functional groups can be brought together and conjugated under favourable conditions (Fig. 4B). Michael-type addition between a nucleophile (i.e. an amine or a thiol) and a vinyl group has also been widely investigated. For example, the strong bonding between the amino groups of chitosan and the carboxyl groups of oxidised alginate forms a hydrogel which can improve the proliferation of olfactory ensheathing cells and NSCs (Wang et al., 2017). A disadvantage of this approach is that the polymer chains require significant modification prior to conjugation (Hoare and Kohane, 2008). Photo-crosslinking with specific wavelengths of light can be used to pattern hydrogelation of polymers with photo-sensitive functional groups (Fig. 4C). A drawback of this technique is that it requires the use of a secondary photosensitiser and prolonged irradiation, both of which can cause local toxicity (Łukaszczyk et al., 2005; Sabnis et al., 2009). Non-toxic photo-crosslinked chitosan-based hydrogels have also been synthesised and their low storage modulus, simulating soft CNS environments, was found to facilitate neuronal differentiation and neurite outgrowth. (Rickett et al., 2011; Valmikinathan et al., 2012). Enzymatic crosslinking occurs when enzymes, such as transglutaminase (Broguiere et al., 2016; Yung et al., 2007) and peroxidase (Wang et al., 2010) catalyse crosslinking reactions between polymer chains. This approach has been used to form fast gelling, non-toxic, stable hydrogels (Teixeira et al., 2012) (Fig. 4D). For example, a high molecular weight HA hydrogel crosslinked with transglutaminase was shown to support fast neurite outgrowth with functional synaptic generation and electrical connectivity between neurons (Broguiere

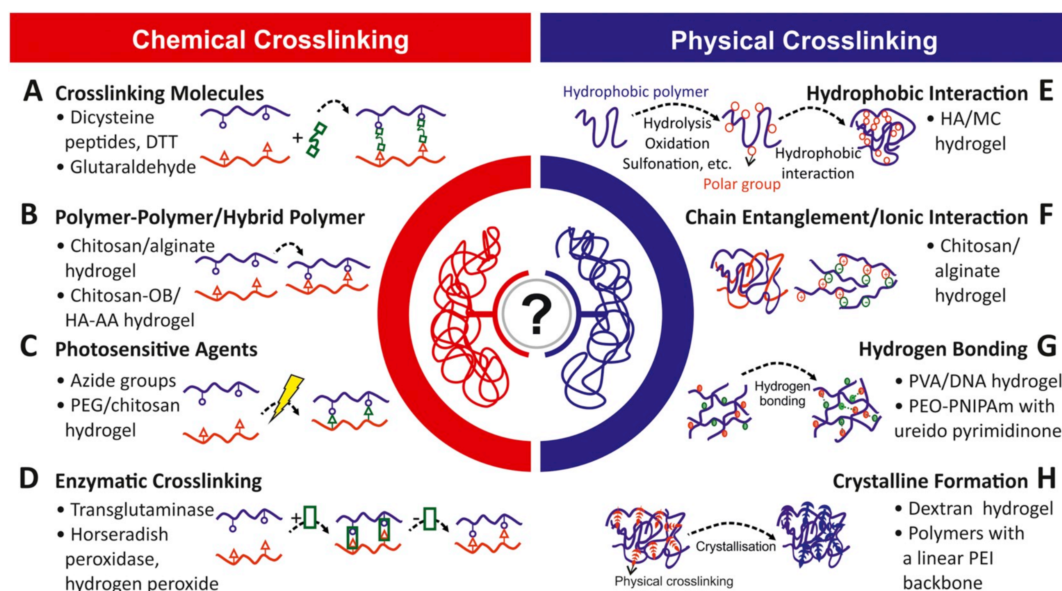

Fig. 4. Methods of hydrogel synthesis. Examples of chemical crosslinking methods and physical crosslinking methods are listed. (A) Dicysteine peptides and dithiothreitol (DTT) crosslinkers were used to crosslink a PEG-based hydrogel for NPC delivery (Zhao et al., 2017a) while glutaraldehyde was used to crosslink a chitosan-poly(vinyl alcohol) (PVA) hydrogel (Wang et al., 2004). (B) Hydrogels made by polymer-polymer interactions, such as a chitosan/alginate hydrogel (Wang et al., 2017) and a hydrogel based on chitosan and hyaluronic acid (HA) modified with oxanorbornadiene (OB) and 11-azido-3,6,9-trioxaundecan-1-amine (AA), respectively (Fan et al., 2015). (C) Photocrosslinked hydrogels based on photoactive azide moieties (Ono et al., 2000; Rickett et al., 2011) and poly(ethylene glycol) (PEG) (Burdick et al., 2006; Piantino et al., 2006) or chitosan (Valmikinathan et al., 2012) based materials can be used for neural tissue engineering applications. (D) Transglutaminase has been used to crosslink hyaluronan (Broguiere et al., 2016) and gelatin hydrogels (Yung et al., 2007) while the combination of horseradish peroxidase and hydrogen peroxide has been used for gelatin-chitosan hydrogel crosslinking (Linh et al., 2017). Physical crosslinking such as (E) hydrophobic interaction and (F) chain entanglement and ionic interaction have been used to synthesise HA/methylcellulose (MC) hydrogels (Baumann et al., 2010; Gupta et al., 2006) and chitosan/alginate hydrogels (Wang et al., 2017), respectively. (G) Hydrogen bonding was used for hydrogelation of a poly(vinyl alcohol) (PVA)/deoxyribonucleic acid (DNA) hydrogel (Kimura et al., 2007) and a hydrogel based on poly(ethylene oxide)-poly(N-isopropylacrylamide) (PEO-PNIPAm) (Zhang et al., 2017). (H) Crystalline formation participates in formation of dextran hydrogels (Stenekes et al., 2001) and hydrogels based on polymers with a poly(ethyleneimine) (PEI) backbone (Yuan and Jin, 2005).

et al., 2016).

Physical crosslinking can be used to form non-covalently crosslinked hydrogels, stabilised through physical chain interaction. Polymer amphiphiles possessing both hydrophilic and hydrophobic domains form self-assembling peptide hydrogels through hydrophobic interaction (Fig. 4E). They have been widely used for neural tissue engineering applications to support attachment and differentiation of neural cells, neurite outgrowth and formation of functional synaptic connections (Gelain et al., 2006; Holmes et al., 2000; Song et al., 2011). Nevertheless, these hydrogels often acquire poor mechanical properties due to the weak nature of this type of crosslinking. Hydrogels can also be physically crosslinked through a process of entanglement or through indirect ionic interactions (Fig. 4F). In one approach, carboxylate groups of sodium alginate and amino groups distributed along chitosan chains were linked through ionic interactions in their polyelectrolyte complexes, and the resulting hydrogels supported the growth of olfactory ensheathing cells and NSC proliferation (Wang et al., 2017). Nevertheless, the stability of ionically crosslinked hydrogels can be affected in physiological conditions through loss or exchange of ions (Matyash et al., 2014). Hydrogen bonding also participates in hydrogel formation in polymers composed of a multitude of hydroxyl groups (Fig. 3G). Such hydrogels are usually tough compared to other physically crosslinked hydrogels (Song et al., 2013; Zhang et al., 2017). Poly (vinyl alcohol)/poly(acrylic acid) hydrogels formed by hydrogen bonding have been used to coat neural electrodes to mitigate inflammatory effects and enhance neurite extension (Lu et al., 2009). Crystalline formation depends on the polymer chain architectures (Fig. 4H). Cellulose chains spatially orientate to form hydrogels based on a composite of two crystalline phases, Iα and Iβ, forming microfibril structures (Koyama et al., 1997), and addition of graphene oxide has been used to modify crystallinity, modulating mechanical properties and porosity of the resulting hydrogel (D. Kim et al., 2017a).

While there are various crosslinking methods for hydrogel synthesis, the choice of crosslinking strategy is often chosen based on the chemistry of the raw material and the desired functionality. In general, physically crosslinked hydrogels prepared at mild and physiologically relevant conditions preserve cell viability better (Hennink and van Nostrum, 2012). Nevertheless, the mechanical properties of physically cross-linked hydrogels tend to be weak, increasing the likelihood of matrix degradation in longer term cultures. Hydrogels modified through chemical crosslinking can provide a more stable substrate, supporting longer culture periods. The crosslinking reagents and the environment of the reaction need to be carefully optimised to support cell incorporation and biocompatibility. Furthermore, the mesh sizes of these hydrogels are generally at the nanometre-scale and this restrict cellular responses, including spreading, proliferation, migration and differentiation (Wang and Heilshorn, 2015).

2.2. *Hydrogel bulk structure (2nd order)*

The physical properties of the polymer chains and the conditions encountered during gelation modulate the internal structure of the hydrogel. This affects gel density, porosity and mechanical properties such as stiffness. In particular, hydrogel stiffness has been reported to modify neuronal cell shape, expression, migration, differentiation and viability (Engler et al., 2006; Saha et al., 2008). Whilst stiffness can be measured as a bulk property of a hydrogel, it is the microscale cellular interaction with the underlying hydrogel structure that leads to modification of cell behaviour. Environmental stiffness is translated onto the cell cytoskeleton via membrane binding and modulates cell behaviour (Handorf et al., 2015) and axonal pathfinding (Koser et al., 2016) during development.

ARTICLE IN PRESS

Choice of hydrogel material and matrix mechanical properties directly modulate the size and number of focal adhesions in a material dependent manner (Charrier et al., 2018). Stiffer synthetic matrices support the growth of focal adhesions through interaction with the stable hydrogel matrix, whilst weaker natural fibrous hydrogels support the growth of focal adhesions through local matrix remodelling (Cao et al., 2017). Focal adhesion size directly modulates the formation of internal cytoskeleton filaments, with larger bundles of filaments leading to increased cell contractility and internal signalling (Wozniak et al., 2004). When cells fail to bind to the hydrogel scaffold, cells adopt a rounded morphology. As cells proliferate locally, cell-to-cell binding can dominate and lead to the formation of dense cell clusters.

Neurons and astrocytes extend neurite processes in all directions, seeking integrin and cadherin binding sites (Tomaselli et al., 1988). The local porosity of the hydrogel in relation to the size of cellular processes will act to restrict or permit cell migration. Neurites range in size and typically have submicron diameters, although mature axons can enlarge to 9 µm in diameter (Liewald et al., 2014). Neuron migration is also constrained by pore size, and track-etched membranes with pore diameters smaller than 2 µm have been shown to block neuron migration (Bružauskaitė et al., 2016; George et al., 2018). Migration in hydrogels is dependent on a complex interplay between the cell's ability to attach to the hydrogel matrix, grow focal adesions and form contractile cytoskeleton filaments, and the hydrogel's physical structure exerted through the constricting role of matrix pore size and pore deformability. Pore size also has an effect on hydrogel perfusion, with smaller pores restricting diffusion. For example, by mixing D-mannitol crystals with photo-crosslinkable methacrylamide chitosan and subsequently dissolving the crystals to increase pore size throughout the hydrogel matrix, it was demonstrated that faster diffusion occured in more porous scaffolds (Li et al., 2012).

Soluble factors such as cytokines, growth factors and trophic factors participate in the fine-tuning of cell behaviour from an early developmental stage to adult CNS (Johe et al., 1996). Delivery of soluble factors can be used to replicate the local expression of supporting cells and more closely model aspects of the in-vivo CNS environment. It is often benifical to control the release rate and the local concentration of bioactive factors. The release rate of incorporated soluble factors is often inversely proportional to a hydrogel's crosslinking density. For example, higher crosslinking densities can result in dense polymer networks that have decreased porosity, slowing the release of incorporated molecules (Hoare and Kohane, 2008), and it has been shown that the release of larger macromolecules is hindered by decreased pore size (Weber et al., 2009). Tailoring the physical properties of hydrogels by modifying the charge density and hydrophobicity of the polymer chains also affects the binding and release of biomolecules while maintaining crosslinking density (Kim and Cha, 2018). Heparin, a highly anionic charged GAG that is abundant in the CNS, possesses a high affinity to a wide variety of important biomolecules (Capila and Linhardt, 2002). Covalent functionalisation of heparin into a PEG hydrogel along with non-covalent loading and release of FGF-2 was found to result in enhanced proliferation, differentiation, and neurite outgrowth of encapsulated NSCs (Freudenberg et al., 2009). Charged hydrogels can be fabricated by mixing or chemically conjugating hydrogel precursors with charged molecules such as polycationic chitosan, polyanionic alginate, and [2-(methacryloyloxy) ethyl]-trimethylammonium chloride (MAETAC) (Dadsetan et al., 2009; Zuidema et al., 2011), and positively charged hydrogels have been shown to support better cell attachment and neurite extension (Crompton et al., 2007; Dadsetan et al., 2009).

Hydrogel systems can be modified to have enzyme-sensitivity, supporting cell-modulated enzymatic degradation and bioresponsive drug release. Matrix metalloproteinases (MMPs), which cleave ECM components, are essential for ECM degradation and remodelling in-vivo, and can also be harnessed for use in hydrogel systems (Ulijn et al., 2007). For example, short peptides specific to MMP degradation have been incorporated into hydrogel systems for delivery of Interleukin-1 receptor antagonist (IL-1Ra) (Gutowski et al., 2015). As MMPs upregulate in inflamed brain tissue, the hydrogel degrades to release IL-1Ra, modulating inflammation and enhancing neuron survival. Peptides sensitive to plasmin have also been incorporated into a PEG-based microsphere system to support the release of GDNF (Roam et al., 2015).

Hydrogel chemistry can be customised to approximate the mechanical properties of the brain, often resulting in soft, weak hydrogels which replicate the elastic modulus of neural tissue which is relatively low compared to other tissues. The storage moduli (G') of the grey matter in an adult human brain has been recorded as ~3.1 kPa and the white matter as ~2.7 kPa, measured by magnetic resonance elastography (Green et al., 2008), whilst the storage moduli of developing brain tissue is significantly lower. The elastic modulus of a human spinal cord is 40 kPa under the tensile load and 89kPa under the compressive load (Karimi et al., 2017). Substrate elasticity can also exert effects on cell behaviours and cell fates. On soft substrates (E = 0.1-1 kPa) hMSCs have been found to exhibit neurogenic profiles, and on soft gels (E = ~0.1-0.5 kPa) adult NSCs exhibit a higher degree of neuronal differentiation whereas stiffer gels (E = ~1-10 kPa) have been found to enhance glial cell differentiation (Engler et al., 2006; Saha et al., 2008). Different methods have been used to modulate hydrogel elasticity and control neural cell behaviour, including varying the concentrations of hydrogel monomers, macromers, ions, salts and crosslinkers, as well as including glycosaminoglycans, which play a role in ECM rigidity and water sorption (Broguiere et al., 2016; Her et al., 2013; Matyash et al., 2014; Palazzolo et al., 2015; Zhou et al., 2016). For example, adult NSCs were cultured in alginate hydrogels made with different concentrations of $CaCl_2$ and the highest degree of cell proliferation and neuronal differentiation was found within the weakest hydrogel with the lowest modulus of 0.183 kPa (Banerjee et al., 2009). Varying the concentration of the polymer precursor modulates gelation time and cell-hydrogel interactions, an effect that is enhanced when polymer chains incorporate bioactive ligands (Wang et al., 2010). For example, enzymatically crosslinked hydrogel from gelatin-hydroxyphenylpropionic acid conjugates was formed with hydrogen peroxide (H_2O_2, the oxidant) and horseradish peroxidase (HRP, the enzyme) catalysts (Wang et al., 2010). Varying the concentration of H_2O_2 and HRP was used to independently control the crosslinking density and the gelation rate respectively, facilitating the fabrication of injectable hydrogels with tunable mechanical properties (Wang et al., 2010).

2.3. Multi-phase structure (3rd order)

Beyond internal hydrogel microstructure, the composite organisation of the scaffold becomes relevant. Within CNS tissue, a greater proportion of the microscale tissue organisation can be thought of as granular, with cells binding to cells. This is a colloidal structure, with dense cell packing as the main phase surrounded by proteoglycan rich extracellular space (Kamali-Zare and Nicholson, 2013). It is possible to replicate this architecture directly using densely packed cells, however, without the benefit of a vascular system, perfusion into and diffusion from tissue sections thicker than 200um is decreased, leading to the generation of toxic microenvironments (Asakawa et al., 2010). To overcome this, hydrogels with higher internal porosity and lower cell densities can be used. The internal structure of this bulk hydrogel system needs to be optimised to support adequate cell adhesion and diffusion, whilst also entrapping expressed factors to support the generation of a niche cellular environment.

Alternatively, the hydrogel structure can be modified to take the form of a granular structure, better replicating the structure of brain tissue. This multiphase structure encompasses hydrogel granules surrounded by an ECM rich secondary phase into which cells are seeded. Tedesco, et al. reported on the use of chitosan hydrogel granules to build a brain-on-a-chip model for research into basic neuroscience and drug screening (Tedesco et al., 2018). The cells grown within this

system were able to successfully recapitulate 3D interconnected networks, with electrophysiological functionality measured using microelectrode arrays (MEAs).

Modification of bulk hydrogels into granular systems conveys further advantages. Granular hydrogels readily fluidise when compressed, a property known as shear thinning (Mealy et al., 2018). This increases the injectability of these systems. Furthermore, cells can be mixed homogeneously with fluidised hydrogel granules and the granular structure negates the effect of settling following mixing. The granules also act as a protective medium that supports cell viability during delivery. Granular systems work well as cell carriers. In a proposed stem-cell delivery therapy for traumatic brain injury, a microsphere chitosan hydrogel scaffold functionalised with heparin to bind FGF-2 was shown to support radial glial cell (RGC) delivery when injected into a rat brain contusion model (Skop et al., 2016). Analysis after 3 days revealed that the RGCs remained positive for nestin, indicating that the microspheres supported RGC viability and maintained the cells in a progenitor state.

Granular scaffolds have also been found to facilitate cell infiltration and promote vascularisation in-vivo. In a recent example, a granulated chitosan hydrogel scaffold was implanted into a rat spinal cord injury model and the granular hydrogel was found to elicit a dynamic restorative process, promoting axon outgrowth, vasculature ingrowth and diminishing fibrous glial scarring and the inflammatory response (Chedly et al., 2017). In a similar way, a microsphere hydrogel scaffold was injected into a rat cortical stroke cavity model with the goal of accelerating neural repair (Nih et al., 2017). The scaffold consisted of HA microgel particles functionalised with multiple groups known to support cell binding and promote tissue repair. The scaffold was found to alter poststroke astrogliosis and inflammation, enhancing vascularisation and neural progenitor cell migration into the site of damage (Fig. 5A).

Hydrogel granules and spherical carrier-embedded hydrogels have also been used in other controlled delivery applications (Kandalam et al., 2017; Li and Mooney, 2016). In one example, poly(N-isopropylacrylamide) (PNIPAm) hydrogel granules were used to facilitate cell transplantation of neurons into rat hippocampus (Jgamadze et al., 2015). PNIPAm hydrogel granules have a thermo-switching capability, such that mature neurons can be dissociated from the granules without neurite damage. In use, the granules enhanced cell survival and minimised the number of carriers needed for cell transplant. Unlike cells encapsulated inside bulk hydrogel, many of the mechanical and biochemical properties of granular gels are effectively decoupled. Whilst it is the bulk material of the hydrogel granules that supports the scaffold and maintains space for diffusion, it is the extended interconnected interface structure that occurs between granules that facilitates and localises the exchange of biological factors and cell-cell interactions (Allazetta et al., 2015; Scott et al., 2011a, 2011b; Smith et al., 2012).

2.4. Single axis alignment (4th order)

Hydrogels that contain anisotropic particles aligned along a single axis are promising candidates for use in generating more realistic models of the CNS, as well as for use in therapies that optimise the bridging between damaged areas of the CNS. This degree of organisation can be achieved through alignment of natural fibrous polymers, such as collagen type I where cell interaction with collagen fibre bundles guide cell growth along the direction of alignment (O'Rourke et al., 2017).

To achieve axis alignment, it is possible to generate pre-aligned subdomains, such as collecting aligned electrospun fibres (Hodde et al., 2016; Lee et al., 2016; McMurtrey, 2014; Schuh et al., 2014). It is also possible to use subdomains which respond to dynamic alignment, such as microgels and electrosun fibres containing superparamagnetic iron oxide nanoparticles and electrospun shape-memory meshes (Omidinia-Anarkoli et al., 2017; Rose et al., 2017; Wang et al., 2018). Using this appraoch, tissue engineered scaffolds possessing non-aligned micro- and nano-domains can be transform into desired aligned structures in response to remote stimuli or through innate recovery, facilitating in-vivo transplantation with minimum-invasive methods (Lee et al., 2016; Wang et al., 2018).

Alignment of neural cells across a hydrogel is also achievable by including cell-sized aligned components within the amorphous hydrogel phase. Biofunctionalised PEG microgels containing magnetic particles have been moulded into cell-sized rods (5x5x50um) and crosslinked using UV light (Rose et al., 2017). When these microgels were mixed with neurons in an amorphous fibrin gel and the microgels were aligned using a millitesla magnetic field, the neurons bound and aligned with the microgels (Fig. 5B-C). Similar results have also been obtained using magneto-responsive cell adhesive short fibres (Omidinia-Anarkoli et al., 2017).

In a further application of single axis alignment, hydrogel channels and conduits have been designed to replicate the form of white matter tracts, possessing long tubular structures assembled by axon bundles. For example nerve conduits with single or multiple guidance channels for axon tract repair have been fabricated using moulding and fibre templating techniques (Struzyna et al., 2015; Dalton et al., 2002; Flynn et al., 2003; Harris et al., 2016; Tsai et al., 2006; Yu and Shoichet, 2005). While conventional moulding methods utilise combinations of moulds and cylindrical tools, such as drills, needles and wires to fabricate nerve conduit channels, fibre templating techniques provide a facile and effective approach to produce highly reproducible scaffolds (Flynn et al., 2003). For example, polycaprolactone (PCL) fibres have been embedded in poly(2-hydroxyethyl methacrylate) (pHEMA) gels and then dissolved by sonicating the pHEMA/PCL composite in acetone. By tuning the size and quantity of the PCL fibres, the diameter and number of channels of the hydrogel conduit can be fine-tuned to have well controlled dimensions (Flynn et al., 2003).

Recently, microfluidic systems have also been used to fabricate tubular hydrogel constructs for culture of aligned nerve fibres (Choi et al., 2011; Hu et al., 2010; Kato-Negishi et al., 2017). In one example, neural tissue was cut into millimetre sized pieces and encapsulated in a tubular alginate hydrogel scaffold. This was placed into a microfluidic device with a three-layer system of coaxial laminar flow (core stream, shell stream, and sheath stream) (Kato-Negishi et al., 2017). The resulting rod-shaped neural units formed synaptic connections and were used to model complex neural connections in-vitro. Aligned subcellular nano- or micro- textures can also be used to template the assembly of multimodal structures. Aligned hydrogels made using microfluidic devices have been developed to support the growth of aligned 3D neural circuits (Bang et al., 2016; Jang et al., 2015). By varying the prepolymer composition (concentration and gelation time), flow rate, and surface modification (hydrophobicity and charging, etc.), it has been possible to create well-aligned and fine-tuned hydrogel scaffolds that support the formation of spatially defined neuronal networks (Lanfer et al., 2008).

2.5. Multi-axis patterning (5th order)

A number of techniques have been used to create multi-axis patterned tissue, achieving patterning at different resolutions. One way to achieve this is to mould, shape or deposit a pattern in a layer-by-layer form that represents a basic tissue structure into which cells are seeded. In one such model, hydrogel layers were added using a syringe and the degree of crosslinking in each layer was increased to create a density gradient migration model representative of tissue structure (Karpiak et al., 2012; Zhang et al., 2016). In another example, physically assembled concentric rings of silk fibre scaffold were impregnated with collagen gel and seeded with primary cortical neurons to create a compartmentalised neural architecture (Tang-Schomer et al., 2014). Neural networks were maintained for months in culture in this system and developed electrophysiological function, enabling their non-destructive use as a mechanical injury model. Decreasing in scale, it is

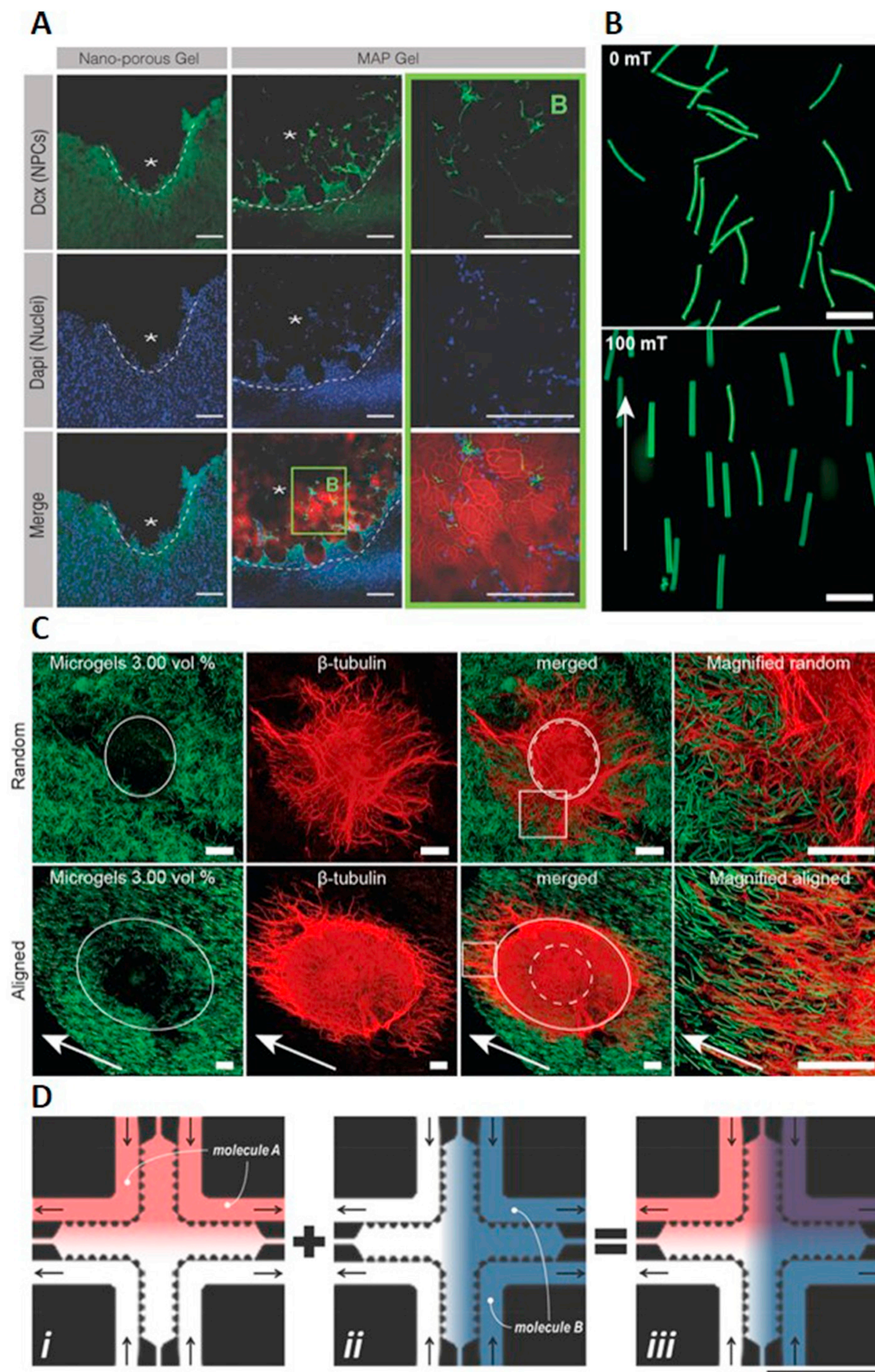

Fig. 5. Examples of structured hydrogels. (A) Nih et al. developed injectable microsphere hydrogels, termed microporous annealed particle (MAP) hydrogels for poststroke brain repair. Endogenous neural progenitor cells (NPCs) migrated from the subventricular zone to the stroke area (scale bars: 0.5 mm). Compared to the nanoporous gel, the MAP gel attracted NPC infiltration into the lesion site and may be able to ameliorate the brain repair processes (Green B; scale bars: 100 μm). Reproduced with permission from (Nih et al., 2017), Copyright © 2017, John Wiley and Sons. (B) Microgels have been doped with superparamagnetic iron oxide nanoparticles that can be subsequently aligned in a 100mT magnetic field (scale bars: 50 μm). Reproduced with the permission from (Rose et al., 2017), Copyright © 2017, American Chemical Society. (C) Dorsal root ganglions in hydrogels with 3% vol SPION-doped microgels (green, fluorescein) exhibited neurite alignment when exposed to an external magnetic field (red, β-tubulin staining; scale bars: 200μm). Reproduced with the permission from (Rose et al., 2017), Copyright © 2017, American Chemical Society. (D) A microfluidic design that can be used to generate diffusion-driven orthogonal linear concentration gradients, such as retinoic acid and smoothened agonist, within a collagen matrix (arrows indicate the direction of flow; scale bars: 5 mm). Reproduced with the permission from (Uzel et al., 2016), Copyright © 2015, John Wiley and Sons.

possible use a microfluidic approach to create multilayer scaffolds that replicate the thickness of human cortical layers. Primary neural cultures were fabricated in layers 100 μm high by 160 μm wide (Kunze et al., 2011), and used to investigate neurite growth between the layers in response to hydrogel stiffness and the inclusion of B27 as a neurite chemoattractant. Whilst microfluidic fabrication was used to patterned hydrogel structure along a single axis, B27 gradients set up inside the system patterned neurite extension across the layers.

The development of 3D bioprinting techniques enables the rapid prototyping of precisely patterned hydrogel structure that can be used to encapsulate and spatially order cells, with a typical feature resolution of 100-200 μm. The field of 3D bioprinting is undergoing rapid development, and for further information, we refer the reader to other recent reviews (Jungst et al., 2016; Knowlton et al., 2018). Weak gels are more permissive to neurite extension and gels with a stiffness of less than 500Pa have typically been used. Advances such as the ability to print weak hydrogel materials into a bath of granular gel (Bhattacharjee et al., 2015) open up new possibilities for building CNS models. During development the CNS is organised spatially via cell position and neurite outgrowth in a process templated by the close and highly ordered organisation of cells and fibres. Replicating this organisation in 3D printed tissues is challenging and any attempt would requires the patterning of additional structures into the hydrogel via the printing of multiple materials (Rocca et al., 2018) with different mechanical and biological properties. Whilst the 3D bioprinting of cellular constructs allows for the generation of arbitrarily complex static tissue environments, these printed constructs can only ever act as a guiding snapshot of tissue state. Ultimately, it is the cells of the tissue that need to grow

together to establish the relevant dynamic internal microstructure and function of the tissue, namely, the tissue's developmental context. During the establishment of developmental context and tissue homeostasis, it is important to recognise the role played by extrinsic factors.

2.6. Extrinsic factors (6th order)

As well as responding to the organisation of the surrounding cellular and extracellular environment, the cells of the developing CNS tissue are sensitive to a multitude of signalling gradients that act to trigger cell differentiation and pattern the ultimate location of neurons and glial cells. The gradients arise as dynamic and spatially patterned concentrations, and external flows can act to damage gradient establishment, such that compartmentalisation of developing CNS tissue is important to preserve patterning gradients. In-vivo, the surrounding basement membrane layer and the establishment of the early blood brain barrier serves to confine fluidic flow and protect the underlying CNS tissue. In-vitro, microbioreactors, microfluidic devices and lab-on-chip systems can be used to compartmentalise culture models, regulating extrinsic input and output to cells in culture. A perfusion system can be used to maintain a steady state baseline flow of medium and biological factors into and out of the culture environment, supporting higher density neural cultures (Cullen et al., 2007). This prevents the build up of toxic concentrations of factors and negates the shock effect where temperature, pH, dissolved gasses and signalling factor levels rapidly flux with the periodic exchange of culture medium.

Beyond the establishment of a baseline level of perfusion, microfluidic devices can be used to recapitulate complex in-vivo gradient profiles with a high level of spatial and dynamic control. It is possible to use a microfluidics approach to recreate the spatial patterning of signalling factors in the neural tube. In one approach, microfluidic organisation of orthogonal gradients of retinoic acid and smoothened agonist were used to stimulate the spatial differentiation of stem cells cultured in a collagen matrix (Uzel et al., 2016) (Fig. 5D). The platform significantly enhanced motor neuron differentiation in regions patterned with high concentrations of both morphogens, replicating in-vivo patterning. Other techniques can also be used to generate concentration gradients, including the use of passive diffusion in static culture (Kunze et al., 2009), slow release from microsphere systems (Lee et al., 2014), and graduated crosslinking methods (Horn-Ranney et al., 2013; Roam et al., 2015). Many factors influence the ability to establish and maintain successful gradients over time, and the reader is referred to recent reviews for further information (Berthier and Beebe, 2014; Sant et al., 2010).

3. Hydrogels in developmental models

Whilst tissue complexity increases throughout development, a core set of underlying mechanisms, tied to a core set of gene expression (Schaefer et al., 2014), is reused and modulated to achieve the different stages of patterning that leads to embryonic organisation as well as tissue function in adulthood. The investigation of in-vitro models of early development allows for better understanding of these principles, allowing study and replication. The use of organoid models has highlighted this approach, and protocols for producing many different types of CNS organoids in healthy and disease states have been investigated (Brawner et al., 2017). The ability to generate organoids has been facilitated by innovation in the availability of stem cells and early stage differentiation protocols. However, it is the use of hydrogels, primarily Matrigel, in these models that helps support the polarisation of surface cells into organised cell sheets that form the basis of downstream organoid patterning.

Matrigel is composed of a mixture of biological components, and a recent study investigated the use of synthetic PEG hydrogel in combination with a library of purified proteins derived from or related to the constituents of Matrigel to generate neuroepithelial cysts from mouse stem cells encapsulated within the hydrogel (Ranga et al., 2016) (Fig. 6A). The study found that 2 kPa stiffness was optimal for the formation of uniform cysts and that the use of Laminin-111 as the adhesive protein was the most effective at reducing variability in formation. However, the use of PEG hydrogel ultimately restricted the growth of the cyst, indicating the need for the encapsulating hydrogel to permit growth of the tissue.

Whereas the neuroepithelial cysts described in the previous study were generated from an encapsulated population of single cells, organoids are typically formed from clusters of 2000 or more cells encapsulated within a drop of Matrigel (Lancaster et al., 2013). Following neural induction, multiple regions similar to the organisation of the neuroepithelial cyst are formed on the surface of the organoid, with the surrounding Matrigel coat providing support in a similar way to basement membrane in-vivo (Fig. 6B). This polarisation of cell sheets replicates the formation of cell sheets within the early embryo (Fig. 6C). Cells within the sheet adopt an apical-lateral-basal polarity with cadherin binding to neighbouring cells towards the apical-luminal side of the cyst and basal integrin binding to the underlying basement membrane. Daughter cells are born in-plane as cells undergo organised mitosis (Nakajima, 2018). In the mature organoid, these neuroepithelial-like sheets undergo developmental patterning similar to early neural tube growth in embryogenesis (Fig. 6D). It was found that as the organoid tissue increased in size, the Matrigel coat weakened, becoming patchy and leading to disorganised regions in the developing basal layer of the sheet (Lancaster et al., 2017). In the early embryo, the neural tube is surrounded by a laminin rich basement membrane (Fig. 6E). Mesodermal cells outside the neural tube continually express basement membrane matrix, reinforcing this membrane layer as the neural tube expands. It was found that addition of Matrigel to the culture medium of the developing organoid could be used to reinforce and increase the integrity of the hydrogel coat, such that the neural progenitor cells remained basally bound to the hydrogel coat (Lancaster et al., 2017). In the neural tube, the RGC soma is apically located and daughter cells that are born in-plane remain as progenitors at the apical face of the tube wall. Whereas many daughter cells born out of plane, towards the basal face, inherit a different set of cytosol components and differentiate, using cell-to-cell binding to migrate up RGC fibres and forming neurons in the cortical plate, switching to form astrocytes and oligodendrocytes in the bulk of the cortex at later stages of development. In this way, the RGCs and RGC fibres together with the basement membrane template the patterning of embryonic growth in the brain. It is likely that this type of patterning is replicated in organoid growth.

On the basal side of the neural tube wall, the basement membrane together with the glial cell end-feet act to compartmentalise the CNS from the surrounding mesoderm tissue, protecting the establishment of gradients of signalling factors within the neural tube, such as the expression of Reelin by early born neural progenitors that acts as a stop signal for later born neurons migrating up RGC fibres (Chai et al., 2009) (Fig. 6D, E). This organisation of the neural tube is carried forwards into adult CNS tissue, with the lumen of the tube forming the ventricular space in the brain and the central canal in the spinal cord, and the basement membrane becoming the richly vascularised pia mater (also known as the pial layer), forming an extensive part of the blood brain barrier and the outer covering of the brain and spine.

Protocols to culture organoid tissues result in variable regional organisation, and a major goal is to develop techniques to better organise early organoid growth. Organoid culture provides a definitive example that complex neural tissue organisation can be generated using a hydrogel system that replicates aspects of the basement membrane in early organogenesis. The opportunity exists to extend current organoid culture techniques using engineered platforms (Roach et al., 2017), and through the use of structured hydrogels, it should be possible to better replicate the early embryonic environment needed to pattern and shape the neural tube. For example, the stiff structure of the notocord plays a fundamental role in templating growth of the early neural tube inside

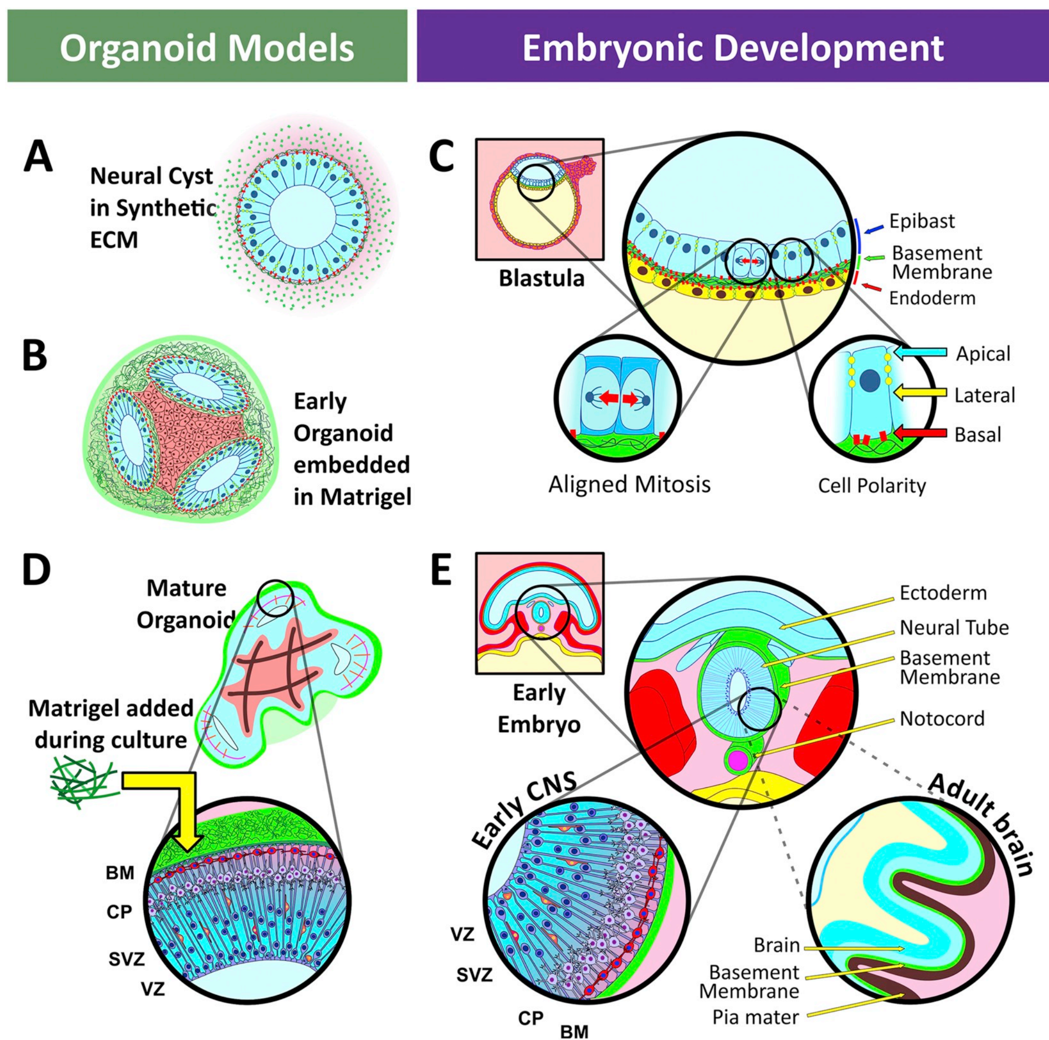

Fig. 6. *Comparing hydrogel developmental models with embryonic development.* (A) Representation of a section through a neuroepithelial cyst, formed from neuralised mouse stem cells suspended as single cells in synthetic PEG hydrogel containing laminin and cultured for 5-7 days (Ranga et al., 2016). The cyst replicates aspects of early neural tube formation. (B) In a similar fashion, cortical organoids embedded in Matrigel recapitulate early cortical organogenesis, albeit with increased variation (Lancaster et al., 2013). (C) Formation of a cell sheet is one of the first patterning events in early embryonic development. Cells within the sheet are polarised, maintaining apical-lateral-basal orientation through cadherin (yellow) and integrin (red) binding. Cells in the epiblast sheet undergo organised aligned mitosis. (D) In the mature organoid, Matrigel supplemented into the medium reinforces the outer basement membrane like layer that supports the attachment of progenitor cells (RGCs), templating the organisation of the early CNS (Lancaster et al., 2017). Reelin expressing neurons (depicted in red) form a band above the cortical plate and express a gradient of reelin (red to blue) into the cortical space. (E) The organoid model recapitulates development in the neural tube, where RGCs become stretched between cadherin mediated cell-to-cell attachment to other RGCs at the lumen of the tube and integrin mediated binding to the basement membrane that surrounds the neural tube. This organisation carries through to the adult brain where the lumen of the neural tube becomes the ventricles of the brain and the outer layer of the neural tube becomes the basement membrane bound by astrocyte end-feet beneath the richly vascular outer pia mater membrane that surrounds the adult CNS. BM: basement membrane; CP: cortical plate; SVZ: subventricular zone; VZ: ventricular zone.

the cellular structure of the mesodermal layer, and it is likely that models that replicate this arrangement will lead to the formation of aligned organoid growth. Generating more realistic healthy and diseased models of the early human CNS is both useful and necessary to elucidate the principles of CNS formation. Furthermore, it is likely that knowledge gained in investigating these types of model will lead directly to the development of treatments that enable the regeneration of lost neural structure.

4. Future perspectives

We are on a journey that will ultimately lead to the discovery of how to recapitulate the developmentally relevant biological patterns needed to replicate and repair the complex tissue microenvironment. Using current techniques it is only becoming possible to generate simple microscale environments such as aligned collagen hydrogels and encapsulation of organoids in basement membrane hydrogel. These simplistic settings can only replicate early aspects of developing tissue architecture. However, this approach is of significant benefit to basic research, where simplified systems are needed to more easily elucidate the complex interactions that occur within biological model systems. It is expected that the use of micro-patterned scaffolds together with compartmental organisation and microfluidic flow will become more widely established, increasing access to advanced tissue models. Accessibility and the ability more easily support interfacing with arrayed sensors and actuators is a further advantage of these types of system.

Of increasing interest is the generation of developmental models that provide protected niche environments to support the growth of neurons in a developmental context. It is expected that through combining advances in structured hydrogels with organoid systems, better control will be gained over organoid variability. Furthermore, the use of

ARTICLE IN PRESS

6th order systems that regulate the delivery of extrinsic factors will enable greater control over extrinsic patterning and bulk transport within organoid tissue models.

Advances in the generation of more structured culture models can be readily translated into regenerative treatments that support the re-establishment of developmental context within tissues. It is expected that this field will move forwards in a stepwise fashion, lagging behind organoid and culture model based enhancements, with granular hydrogels initially used to support tissue viability and aligned patterned hydrogels subsequently used to organise tissue growth. Ultimately, it is expected that the techniques discussed will lead to the ability to robustly pattern the macroscale organisation of implanted cells and materials, and the possibility exists that this approach will lead to the in-vitro development and subsequent implantation of cultured tissue sections that contain and restore developmental context.

5. Conclusions

CNS tissue damage results in the direct loss of neuronal and glial cells as well as indirect damage and loss to tissue structure. Delivery of cells or homogeneous materials into damaged tissues does not restore the lost tissue context, just as unstructured neural models cannot fully replicate physiological neural network function. Whilst the bulk physical properties of hydrogels can be optimised to maximise CNS cell viability, use of multi-phase structure within these hydrogels is needed to guide cell growth and support the development and homeostasis of maroscale CNS physiology.

Restoration of the neural and progenitor cell population in the adult brain and spine is only part of the solution to treating CNS damage. Gaining control over the development of CNS architecture is required to support the generation of effective CNS models and therapies. Advances in many separate fields are moving forwards to create the techniques needed to produce highly organised structured hydrogel systems, and this will open up new possibilities in CNS treatments and fundamental research.

Ultimately, the ability to gain control over physical patterning within engineered tissues and create environments which support the establishment of developmental context will lead to the ability to generate models and therapies that can recover and maintain tissue homeostasis needed to support long term biological function.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Authors acknowledge the financial support from China Regenerative Medicine International Limited (CRMI), Hong Kong and Biotechnology and Biological Sciences Research Council (BB/H008527/1) (www.bbsrc.ac.uk).

References

Abu-Rub, M.T., Billiar, K.L., van Es, M.H., Knight, A., Rodriguez, B.J., Zeugolis, D.I., McMahon, S., Windebank, A.J., Pandit, A., 2011. Nano-textured self-assembled aligned collagen hydrogels promote directional neurite guidance and overcome inhibition by myelin associated glycoprotein. Soft Matter 7, 2770–2781.
Aguilar, J.P., Lipka, M., Primo, G.A., Licon-Bernal, E.E., Fernández-Pradas, J.M., Yaroshchuk, A., Albericio, F., Mata, A., 2018. 3D electrophoresis-assisted lithography (3deal): 3d molecular printing to create functional patterns and anisotropic hydrogels. Adv. Funct. Mater. 28, 1703014.
Akassoglou, K., Yu, W.-M., Akpinar, P., Strickland, S., 2002. Fibrin inhibits peripheral nerve remyelination by regulating Schwann cell differentiation. Neuron 33, 861–875.
Alexander, J.K., Fuss, B., Colello, R.J., 2006. Electric field-induced astrocyte alignment directs neurite outgrowth. Neuron Glia Biol. 2, 93–103.
Allazetta, S., Kolb, L., Zerbib, S., Bardy, J., Lutolf, M.P., 2015. Cell-instructive microgels with tailor-made physicochemical properties. Small 11, 5647–5656.
Amamoto, R., Huerta, V.G.L., Takahashi, E., Dai, G., Grant, A.K., Fu, Z., Arlotta, P., 2016. Adult axolotls can regenerate original neuronal diversity in response to brain injury. Elife 5. https://doi.org/10.7554/eLife.13998.
Amsden, B., 1998. Solute diffusion within hydrogels. mechanisms and models. Macromolecules 31, 8382–8395.
Ansorena, E., De Berdt, P., Ucakar, B., Simón-Yarza, T., Jacobs, D., Schakman, O., Jankovski, A., Deumens, R., Blanco-Prieto, M.J., Préat, V., Des Rieux, A., 2013. Injectable alginate hydrogel loaded with GDNF promotes functional recovery in a hemisection model of spinal cord injury. Int. J. Pharm. 455, 148–158.
Antman-Passig, M., Shefi, O., 2016. Remote magnetic orientation of 3D collagen hydrogels for directed neuronal regeneration. Nano Lett. 16, 2567–2573.
Asakawa, N., Shimizu, T., Tsuda, Y., Sekiya, S., Sasagawa, T., Yamato, M., Fukai, F., Okano, T., 2010. Pre-vascularization of in vitro three-dimensional tissues created by cell sheet engineering. Biomaterials 31, 3903–3909.
Aurand, E.R., Wagner, J., Lanning, C., Bjugstad, K.B., 2012. Building biocompatible hydrogels for tissue engineering of the brain and spinal cord. J. Funct. Biomater. 3, 839–863.
Aurand, E.R., Wagner, J.L., Shandas, R., Bjugstad, K.B., 2014. Hydrogel formulation determines cell fate of fetal and adult neural progenitor cells. Stem Cell Res. 12, 11–23.
Banerjee, A., Arha, M., Choudhary, S., Ashton, R.S., Bhatia, S.R., Schaffer, D.V., Kane, R.S., 2009. The influence of hydrogel modulus on the proliferation and differentiation of encapsulated neural stem cells. Biomaterials 30, 4695–4699.
Bang, S., Na, S., Jang, J.M., Kim, J., Jeon, N.L., 2016. Engineering-aligned 3D neural circuit in microfluidic device. Adv. Healthc. Mater. 5, 159–166.
Banwell, E.F., Abelardo, E.S., Adams, D.J., Birchall, M.A., Corrigan, A., Donald, A.M., Kirkland, M., Serpell, L.C., Butler, M.F., Woolfson, D.N., 2009. Rational design and application of responsive alpha-helical peptide hydrogels. Nat. Mater. 8, 596–600.
Baumann, M.D., Kang, C.E., Tator, C.H., Shoichet, M.S., 2010. Intrathecal delivery of a polymeric nanocomposite hydrogel after spinal cord injury. Biomaterials 31, 7631–7639.
Berthier, E., Beebe, D.J., 2014. Gradient generation platforms: new directions for an established microfluidic technology. Lab Chip 14, 3241–3247.
Bhattacharjee, T., Zehnder, S.M., Rowe, K.G., Jain, S., Nixon, R.M., Sawyer, W.G., Angelini, T.E., 2015. Writing in the granular gel medium. Sci. Adv. 1 e1500655.
Bible, E., Dell'Acqua, F., Solanky, B., Balducci, A., Crapo, P.M., Badylak, S.F., Ahrens, E.T., Modo, M., 2012. Non-invasive imaging of transplanted human neural stem cells and ECM scaffold remodeling in the stroke-damaged rat brain by (19)F- and diffusion-MRI. Biomaterials 33, 2858–2871.
Brawner, A.T., Xu, R., Liu, D., Jiang, P., 2017. Generating CNS organoids from human induced pluripotent stem cells for modeling neurological disorders. Int. J. Physiol. Pathophysiol. Pharmacol. 9, 101–111.
Broguiere, N., Isenmann, L., Zenobi-Wong, M., 2016. Novel enzymatically cross-linked hyaluronan hydrogels support the formation of 3D neuronal networks. Biomaterials 99, 47–55.
Bružauskaitė, I., Bironaitė, D., Bagdonas, E., Bernotienė, E., 2016. Scaffolds and cells for tissue regeneration: different scaffold pore sizes—different cell effects. Cytotechnology 68, 355–369.
Burdick, J.A., Prestwich, G.D., 2011. Hyaluronic acid hydrogels for biomedical applications. Adv. Mater. 23, H41–H56.
Burdick, J.A., Ward, M., Liang, E., Young, M.J., Langer, R., 2006. Stimulation of neurite outgrowth by neurotrophins delivered from degradable hydrogels. Biomaterials 27, 452–459.
Caliari, S.R., Burdick, J.A., 2016. A practical guide to hydrogels for cell culture. Nat. Methods 13, 405.
Cao, X., Ban, E., Baker, B.M., Lin, Y., Burdick, J.A., Chen, C.S., Shenoy, V.B., 2017. Multiscale model predicts increasing focal adhesion size with decreasing stiffness in fibrous matrices. Proc. Natl. Acad. Sci. U. S. A. 114, E4549–E4555.
Capila, I., Linhardt, R.J., 2002. Heparin–protein interactions. Angew. Chem. Int. Ed. 41, 390–412.
Ceballos, D., Navarro, X., Dubey, N., Wendelschafer-Crabb, G., Kennedy, W.R., Tranquillo, R.T., 1999. Magnetically aligned collagen gel filling a collagen nerve guide improves peripheral nerve regeneration. Exp. Neurol. 158, 290–300.
Chai, X., Förster, E., Zhao, S., Bock, H.H., Frotscher, M., 2009. Reelin acts as a stop signal for radially migrating neurons by inducing phosphorylation of n-cofilin at the leading edge. Commun. Integr. Biol. 2, 375–377.
Charrier, E.E., Pogoda, K., Wells, R.G., Janmey, P.A., 2018. Control of cell morphology and differentiation by substrates with independently tunable elasticity and viscous dissipation. Nat. Commun. 9, 449.
Chedly, J., Soares, S., Montembault, A., von Boxberg, Y., Veron-Ravaille, M., Mouffle, C., Benassy, M.-N., Taxi, J., David, L., Nothias, F., 2017. Physical chitosan microhydrogels as scaffolds for spinal cord injury restoration and axon regeneration. Biomaterials 138, 91–107.
Chen, B., He, J., Yang, H., Zhang, Q., Zhang, L., Zhang, X., Xie, E., Liu, C., Zhang, R., Wang, Y., Huang, L., Hao, D., 2015. Repair of spinal cord injury by implantation of bFGF-incorporated HEMA-MOETACL hydrogel in rats. Sci. Rep. 5, 9017.
Cheng, T.-Y., Chen, M.-H., Chang, W.-H., Huang, M.-Y., Wang, T.-W., 2013. Neural stem cells encapsulated in a functionalized self-assembling peptide hydrogel for brain tissue engineering. Biomaterials 34, 2005–2016.
Choi, C.-H., Yi, H., Hwang, S., Weitz, D.A., Lee, C.-S., 2011. Microfluidic fabrication of complex-shaped microfibers by liquid template-aided multiphase microflow. Lab Chip 11, 1477–1483.
Cook, D.J., Nguyen, C., Chun, H.N., Llorente I., L., Chiu, A.S., Machnicki, M., Zarembinski, T.I., Carmichael, S.T., 2017. Hydrogel-delivered brain-derived neurotrophic factor promotes tissue repair and recovery after stroke. J. Cereb. Blood Flow Metab. 37, 1030–1045.
Crapo, P.M., Medberry, C.J., Reing, J.E., Tottey, S., van der Merwe, Y., Jones, K.E., Badylak, S.F., 2012. Biologic scaffolds composed of central nervous system

extracellular matrix. Biomaterials 33, 3539–3547.
Crompton, K.E., Goud, J.D., Bellamkonda, R.V., Gengenbach, T.R., Finkelstein, D.I., Horne, M.K., Forsythe, J.S., 2007. Polylysine-functionalised thermoresponsive chitosan hydrogel for neural tissue engineering. Biomaterials 28, 441–449.
Cullen, D.K., Vukasinovic, J., Glezer, A., Laplaca, M.C., 2007. Microfluidic engineered high cell density three-dimensional neural cultures. J. Neural Eng. 4, 159–172.
Dadsetan, M., Knight, A.M., Lu, L., Windebank, A.J., Yaszemski, M.J., 2009. Stimulation of neurite outgrowth using positively charged hydrogels. Biomaterials 30, 3874–3881.
Dalheim, M.Ø., Vanacker, J., Najmi, M.A., Aachmann, F.L., Strand, B.L., Christensen, B.E., 2016. Efficient functionalization of alginate biomaterials. Biomaterials 80, 146–156.
Dalton, P.D., Flynn, L., Shoichet, M.S., 2002. Manufacture of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogel tubes for use as nerve guidance channels. Biomaterials 23, 3843–3851.
Das, S., Kumar, R., Jha, N.N., Maji, S.K., 2017. Controlled exposure of bioactive growth factor in 3d amyloid hydrogel for stem cells differentiation. Adv. Healthc. Mater. 6. https://doi.org/10.1002/adhm.201700368.
De France, K.J., Xu, F., Hoare, T., 2018. Structured macroporous hydrogels: progress, challenges, and opportunities. Adv. Healthc. Mater. 7. https://doi.org/10.1002/adhm.201700927.
Deister, C., Aljabari, S., Schmidt, C.E., 2007. Effects of collagen 1, fibronectin, laminin and hyaluronic acid concentration in multi-component gels on neurite extension. J. Biomater. Sci. Polym. Ed. 18, 983–997.
DeQuach, J.A., Yuan, S.H., Goldstein, L.S.B., Christman, K.L., 2011. Decellularized porcine brain matrix for cell culture and tissue engineering scaffolds. Tissue Eng. Part A 17, 2583–2592.
Dhoot, N.O., Tobias, C.A., Fischer, I., Wheatley, M.A., 2004. Peptide-modified alginate surfaces as a growth permissive substrate for neurite outgrowth. J. Biomed. Mater. Res. A Soc. Biomater. Aust. Soc. Biomater. Korean Soc. Biomater. 71, 191–200.
DiLuca, M., Olesen, J., 2014. The cost of brain diseases: a burden or a challenge? Neuron 82, 1205–1208.
Dodla, M.C., Bellamkonda, R.V., 2008. Differences between the effect of anisotropic and isotropic laminin and nerve growth factor presenting scaffolds on nerve regeneration across long peripheral nerve gaps. Biomaterials 29, 33–46.
Egawa, E.Y., Kato, K., Hiraoka, M., Nakaji-Hirabayashi, T., Iwata, H., 2011. Enhanced proliferation of neural stem cells in a collagen hydrogel incorporating engineered epidermal growth factor. Biomaterials 32, 4737–4743.
Engler, A.J., Sen, S., Sweeney, H.L., Discher, D.E., 2006. Matrix elasticity directs stem cell lineage specification. Cell 126, 677–689.
Fan, M., Ma, Y., Mao, J., Zhang, Z., Tan, H., 2015. Cytocompatible in situ forming chitosan/hyaluronan hydrogels via a metal-free click chemistry for soft tissue engineering. Acta Biomater. 20, 60–68.
Fitch, M.T., Silver, J., 2008. CNS injury, glial scars, and inflammation: inhibitory extracellular matrices and regeneration failure. Exp. Neurol. 209, 294–301.
Flynn, L., Dalton, P.D., Shoichet, M.S., 2003. Fiber templating of poly(2-hydroxyethyl methacrylate) for neural tissue engineering. Biomaterials 24, 4265–4272.
Fon, D., Al-Abboodi, A., Chan, P.P.Y., Zhou, K., Crack, P., Finkelstein, D.I., Forsythe, J.S., 2014. Effects of GDNF-loaded injectable gelatin-based hydrogels on endogenous neural progenitor cell migration. Adv. Healthc. Mater. 3, 761–774.
Freudenberg, U., Hermann, A., Welzel, P.B., Stirl, K., Schwarz, S.C., Grimmer, M., Zieris, A., Panyanuwat, W., Zschoche, S., Meinhold, D., Storch, A., Werner, C., 2009. A star-PEG–heparin hydrogel platform to aid cell replacement therapies for neurodegenerative diseases. Biomaterials 30, 5049–5060.
Ge, D., Song, K., Guan, S., Qi, Y., Guan, B., Li, W., Liu, J., Ma, X., Liu, T., Cui, Z., 2013. Culture and differentiation of rat neural stem/progenitor cells in a three-dimensional collagen scaffold. Appl. Biochem. Biotechnol. 170, 406–419.
Gelain, F., Bottai, D., Vescovi, A., Zhang, S., 2006. Designer self-assembling peptide nanofiber scaffolds for adult mouse neural stem cell 3-dimensional cultures. PLoS One 1 e119.
George, J.H., Nagel, D., Waller, S., Hill, E., Parri, H.R., Coleman, M.D., Cui, Z., Ye, H., 2018. A closer look at neuron interaction with track-etched microporous membranes. Sci. Rep. 8, 15552.
Gerardo-Nava, J., Hodde, D., Katona, I., Bozkurt, A., Grehl, T., Steinbusch, H.W.M., Weis, J., Brook, G.A., 2014. Spinal cord organotypic slice cultures for the study of regenerating motor axon interactions with 3D scaffolds. Biomaterials 35, 4288–4296.
Gnavi, S., di Blasio, L., Tonda-Turo, C., Mancardi, A., Primo, L., Ciardelli, G., Gambarotta, G., Geuna, S., Perroteau, I., 2017. Gelatin-based hydrogel for vascular endothelial growth factor release in peripheral nerve tissue engineering. J. Tissue Eng. Regen. Med. 11, 459–470.
Grade, S., Götz, M., 2017. Neuronal replacement therapy: previous achievements and challenges ahead. NPJ Regen. Med. 2, 29.
Green, M.A., Bilston, L.E., Sinkus, R., 2008. In vivo brain viscoelastic properties measured by magnetic resonance elastography. NMR Biomed. 21, 755–764.
Gupta, D., Tator, C.H., Shoichet, M.S., 2006. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. Biomaterials 27, 2370–2379.
Gutowski, S.M., Shoemaker, J.T., Templeman, K.L., Wei, Y., Latour Jr., R.A., Bellamkonda, R.V., LaPlaca, M.C., García, A.J., 2015. Protease-degradable PEG-maleimide coating with on-demand release of IL-1Ra to improve tissue response to neural electrodes. Biomaterials 44, 55–70.
Han, Y.L., Wang, S., Zhang, X., Li, Y., Huang, G., Qi, H., Pingguan-Murphy, B., Li, Y., Lu, T.J., Xu, F., 2014. Engineering physical microenvironment for stem cell based regenerative medicine. Drug Discov. Today 19, 763–773.
Handorf, A.M., Zhou, Y., Halanski, M.A., Li, W.-J., 2015. Tissue stiffness dictates development, homeostasis, and disease progression. Organogenesis 11, 1–15.
Harris, J.P., Struzyna, L.A., Murphy, P.L., Adewole, D.O., Kuo, E., Cullen, D.K., 2016. Advanced biomaterial strategies to transplant preformed micro-tissue engineered neural networks into the brain. J. Neural Eng. 13 016019.
Hennink, W.E., van Nostrum, C.F., 2012. Novel crosslinking methods to design hydrogels. Adv. Drug Deliv. Rev. 54, 13–36.
Her, G.J., Wu, H.-C., Chen, M.-H., Chen, M.-Y., Chang, S.-C., Wang, T.-W., 2013. Control of three-dimensional substrate stiffness to manipulate mesenchymal stem cell fate toward neuronal or glial lineages. Acta Biomater. 9, 5170–5180.
Herland, A., van der Meer, A.D., FitzGerald, E.A., Park, T.-E., Sleeboom, J.J.F., Ingber, D.E., 2016. Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip. PLoS One 11 e0150360.
Hoare, T.R., Kohane, D.S., 2008. Hydrogels in drug delivery: progress and challenges. Polymer 49, 1993–2007.
Hodde, D., Gerardo-Nava, J., Wöhlk, V., Weinandy, S., Jockenhövel, S., Kriebel, A., Altinova, H., Steinbusch, H.W.M., Möller, M., Weis, J., Mey, J., Brook, G.A., 2016. Characterisation of cell-substrate interactions between Schwann cells and three-dimensional fibrin hydrogels containing orientated nanofibre topographical cues. Eur. J. Neurosci. 43, 376–387.
Holmes, T.C., de Lacalle, S., Su, X., Liu, G., Rich, A., Zhang, S., 2000. Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds. Proc. Natl. Acad. Sci. U. S. A. 97, 6728–6733.
Hopkins, A.M., De Laporte, L., Tortelli, F., Spedden, E., Staii, C., Atherton, T.J., Hubbell, J.A., Kaplan, D.L., 2013. Silk hydrogels as soft substrates for neural tissue engineering. Adv. Funct. Mater. 23, 5140–5149.
Horn-Ranney, E.L., Curley, J.L., Catig, G.C., Huval, R.M., Moore, M.J., 2013. Structural and molecular micropatterning of dual hydrogel constructs for neural growth models using photochemical strategies. Biomed. Microdevices 15, 49–61.
Hu, M., Deng, R., Schumacher, K.M., Kurisawa, M., Ye, H., Purnamawati, K., Ying, J.Y., 2010. Hydrodynamic spinning of hydrogel fibers. Biomaterials 31, 863–869.
Hughes, C.S., Postovit, L.M., Lajoie, G.A., 2010. Matrigel: a complex protein mixture required for optimal growth of cell culture. Proteomics 10, 1886–1890.
Jang, J.M., Tran, S.-H.-T., Na, S.C., Jeon, N.L., 2015. Engineering controllable architecture in matrigel for 3D cell alignment. ACS Appl. Mater. Interfaces 7, 2183–2188.
Jgamadze, D., Liu, L., Vogler, S., Chu, L.-Y., Pautot, S., 2015. Thermoswitching microgel carriers improve neuronal cell growth and cell release for cell transplantation. Tissue Eng. Part C Methods 21, 65–76.
Jiang, Y., Chen, J., Deng, C., Suuronen, E.J., Zhong, Z., 2014. Click hydrogels, microgels and nanogels: emerging platforms for drug delivery and tissue engineering. Biomaterials 35, 4969–4985.
Johe, K.K., Hazel, T.G., Muller, T., Dugich-Djordjevic, M.M., McKay, R.D., 1996. Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. Genes Dev. 10, 3129–3140.
Jungst, T., Smolan, W., Schacht, K., Scheibel, T., Groll, J., 2016. Strategies and molecular design criteria for 3d printable hydrogels. Chem. Rev. 116, 1496–1539.
Kamali-Zare, P., Nicholson, C., 2013. Brain extracellular space: geometry, matrix and physiological importance. Basic Clin. Neurosci. 4, 282–286.
Kandalam, S., Sindji, L., Delcroix, G.J.-R., Violet, F., Garric, X., André, E.M., Schiller, P.C., Venier-Julienne, M.-C., des Rieux, A., Guicheux, J., Montero-Menei, C.N., 2017. Pharmacologically active microcarriers delivering BDNF within a hydrogel: novel strategy for human bone marrow-derived stem cells neural/neuronal differentiation guidance and therapeutic secretome enhancement. Acta Biomater. 49, 167–180.
Karimi, A., Shojaei, A., Tehrani, P., 2017. Mechanical properties of the human spinal cord under the compressive loading. J. Chem. Neuroanat. 86, 15–18.
Karpiak, J.V., Ner, Y., Almutairi, A., 2012. Density gradient multilayer polymerization for creating complex tissue. Adv. Mater. 24, 1466–1470.
Karve, I.P., Taylor, J.M., Crack, P.J., 2016. The contribution of astrocytes and microglia to traumatic brain injury. Br. J. Pharmacol. 173, 692–702.
Kato-Negishi, M., Onoe, H., Ito, A., Takeuchi, S., 2017. Rod-shaped neural units for aligned 3d neural network connection. Adv. Healthc. Mater. 6. https://doi.org/10.1002/adhm.201700143.
Khaing, Z.Z., Milman, B.D., Vanscoy, J.E., Seidlits, S.K., Grill, R.J., Schmidt, C.E., 2011. High molecular weight hyaluronic acid limits astrocyte activation and scar formation after spinal cord injury. J. Neural Eng. 8 046033.
Kim, D., Park, S., Jo, I., Kim, S.-M., Kang, D.H., Cho, S.-P., Park, J.B., Hong, B.H., Yoon, M.-H., 2017a. Multiscale modulation of nanocrystalline cellulose hydrogel via nanocarbon hybridization for 3D neuronal bilayer formation. Small 13. https://doi.org/10.1002/smll.201700331.
Kim, J., Staunton, J.R., Tanner, K., 2016. Independent control of topography for 3d patterning of the ecm microenvironment. Adv. Mater. 28, 132–137.
Kim, M., Cha, C., 2018. Modulation of functional pendant chains within poly(ethylene glycol) hydrogels for refined control of protein release. Sci. Rep. 8, 4315.
Kim, S.H., Im, S.-K., Oh, S.-J., Jeong, S., Yoon, E.-S., Lee, C.J., Choi, N., Hur, E.-M., 2017b. Anisotropically organized three-dimensional culture platform for reconstruction of a hippocampal neural network. Nat. Commun. 8, 14346.
Kimura, T., Iwai, S., Moritan, T., Nam, K., Mutsuo, S., Yoshizawa, H., Okada, M., Furuzono, T., Fujisato, T., Kishida, A., 2007. Preparation of poly(vinyl alcohol)/DNA hydrogels via hydrogen bonds formed on ultra-high pressurization and controlled release of DNA from the hydrogels for gene delivery. J. Artif. Organs 10, 104–108.
Knowlton, S., Anand, S., Shah, T., Tasoglu, S., 2018. Bioprinting for neural tissue engineering. Trends Neurosci. 41, 31–46.
Koser, D.E., Thompson, A.J., Foster, S.K., Dwivedy, A., Pillai, E.K., Sheridan, G.K., Svoboda, H., Viana, M., Costa, L. Da F., Guck, J., Holt, C.E., Franze, K., 2016. Mechanosensing is critical for axon growth in the developing brain. Nat. Neurosci. 19, 1592–1598.
Kothapalli, C.R., Kamm, R.D., 2013. 3D matrix microenvironment for targeted differentiation of embryonic stem cells into neural and glial lineages. Biomaterials 34, 5995–6007.

ARTICLE IN PRESS

Koyama, M., Helbert, W., Imai, T., Sugiyama, J., Henrissat, B., 1997. Parallel-up structure evidences the molecular directionality during biosynthesis of bacterial cellulose. Proc. Natl. Acad. Sci. U. S. A. 94, 9091–9095.
Kunze, A., Bertsch, A., Giugliano, M., Renaud, P., 2009. Microfluidic hydrogel layers with multiple gradients to stimulate and perfuse three-dimensional neuronal cell cultures. Procedia Chemistry 1, 369–372.
Kunze, A., Giugliano, M., Valero, A., Renaud, P., 2011. Micropatterning neural cell cultures in 3D with a multi-layered scaffold. Biomaterials 32, 2088–2098.
Kuo, Y.-C., Chang, Y.-H., 2013. Differentiation of induced pluripotent stem cells toward neurons in hydrogel biomaterials. Colloids Surf. B Biointerfaces 102, 405–411.
Lampe, K.J., Mooney, R.G., Bjugstad, K.B., Mahoney, M.J., 2010. Effect of macromer weight percent on neural cell growth in 2D and 3D nondegradable PEG hydrogel culture. J. Biomed. Mater. Res. A 94, 1162–1171.
Lancaster, M.A., Corsini, N.S., Wolfinger, S., Gustafson, E.H., Phillips, A.W., Burkard, T.R., Otani, T., Livesey, F.J., Knoblich, J.A., 2017. Guided self-organization and cortical plate formation in human brain organoids. Nat. Biotechnol. 35, 659–666.
Lancaster, M.A., Renner, M., Martin, C.-A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., Knoblich, J.A., 2013. Cerebral organoids model human brain development and microcephaly. Nature 501, 373–379.
Lanfer, B., Freudenberg, U., Zimmermann, R., Stamov, D., Körber, V., Werner, C., 2008. Aligned fibrillar collagen matrices obtained by shear flow deposition. Biomaterials 29, 3888–3895.
Lee, J.H., Yu, H.S., Lee, G.S., Ji, A., Hyun, J.K., Kim, H.W., 2011. Collagen gel three-dimensional matrices combined with adhesive proteins stimulate neuronal differentiation of mesenchymal stem cells. J. R. Soc. Interface 8, 998–1010.
Lee, S., Yun, S., Park, K.I., Jang, J.-H., 2016. Sliding fibers: slidable, injectable, and gel-like electrospun nanofibers as versatile cell carriers. ACS Nano 10, 3282–3294.
Lee, W., Frank, C.W., Park, J., 2014. Directed axonal outgrowth using a propagating gradient of IGF-1. Adv. Mater. 26, 4936–4940.
Lee, Y.-B., Polio, S., Lee, W., Dai, G., Menon, L., Carroll, R.S., Yoo, S.-S., 2010. Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture. Exp. Neurol. 223, 645–652.
Leipzig, N.D., Wylie, R.G., Kim, H., Shoichet, M.S., 2011. Differentiation of neural stem cells in three-dimensional growth factor-immobilized chitosan hydrogel scaffolds. Biomaterials 32, 57–64.
Li, H., Ham, T.R., Neill, N., Farrag, M., Mohrman, A.E., Koenig, A.M., Leipzig, N.D., 2016. A hydrogel bridge incorporating immobilized growth factors and neural stem/progenitor cells to treat spinal cord injury. Adv. Healthc. Mater. 5, 802–812.
Li, H., Koenig, A.M., Sloan, P., Leipzig, N.D., 2014. In vivo assessment of guided neural stem cell differentiation in growth factor immobilized chitosan-based hydrogel scaffolds. Biomaterials 35, 9049–9057.
Li, H., Wijekoon, A., Leipzig, N.D., 2012. 3D differentiation of neural stem cells in macroporous photopolymerizable hydrogel scaffolds. PLoS One 7 e48824.
Li, H., Zheng, J., Wang, H., Becker, M.L., Leipzig, N.D., 2018. Neural stem cell encapsulation and differentiation in strain promoted crosslinked polyethylene glycol-based hydrogels. J. Biomater. Appl. 32, 1222–1230.
Li, J., Mooney, D.J., 2016. Designing hydrogels for controlled drug delivery. Nat. Rev. Mater. 1. https://doi.org/10.1038/natrevmats.2016.71.
Liewald, D., Miller, R., Logothetis, N., Wagner, H.-J., Schüz, A., 2014. Distribution of axon diameters in cortical white matter: an electron-microscopic study on three human brains and a macaque. Biol. Cybern. 108, 541–557.
Lin, C.-M., Lin, J.-W., Chen, Y.-C., Shen, H.-H., Wei, L., Yeh, Y.-S., Chiang, Y.-H., Shih, R., Chiu, P.-L., Hung, K.-S., Yang, L.-Y., Chiu, W.-T., 2009. Hyaluronic acid inhibits the glial scar formation after brain damage with tissue loss in rats. Surg. Neurol. 72 (Suppl. 2), S50–S54.
Linh, N.T.B., Abueva, C.D.G., Lee, B.-T., 2017. Enzymatic in situ formed hydrogel from gelatin-tyramine and chitosan-4-hydroxylphenyl acetamide for the co-delivery of human adipose-derived stem cells and platelet-derived growth factor towards vascularization. Biomed. Mater. 12 015026.
Liu, L., Liu, Y., Li, J., Du, G., Chen, J., 2011. Microbial production of hyaluronic acid: current state, challenges, and perspectives. Microb. Cell Factories 10, 99.
Lozano, R., Stevens, L., Thompson, B.C., Gilmore, K.J., Gorkin III, R.A., Stewart, E.M., Romero-Ortega, M.I., Wallace, G.G., Others, 2016. Brain on a bench top.
Lu, Y., Wang, D., Li, T., Zhao, X., Cao, Y., Yang, H., Duan, Y.Y., 2009. Poly(vinyl alcohol)/poly(acrylic acid) hydrogel coatings for improving electrode-neural tissue interface. Biomaterials 30, 4143–4151.
Luckenbill-Edds, L., 1997. Laminin and the mechanism of neuronal outgrowth. Brain Res. Brain Res. Rev. 23, 1–27.
Lühmann, T., Hall, H., 2009. Cell guidance by 3D-gradients in hydrogel matrices: importance for biomedical applications. Materials 2, 1058–1083.
Łukaszczyk, J., Śmiga, M., Jaszcz, K., Adler, H.P., Jähne, E., Kaczmarek, M., 2005. Evaluation of oligo (ethylene glycol) dimethacrylates effects on the properties of new biodegradable bone cement compositions. Macromol. Biosci. 5, 64–69.
Luo, Y., Shoichet, M.S., 2004. A photolabile hydrogel for guided three-dimensional cell growth and migration. Nat. Mater. 3, 249–253.
Ma, S., Yu, B., Pei, X., Zhou, F., 2016. Structural hydrogels. Polymer 98, 516–535.
Mahoney, M.J., Anseth, K.S., 2006. Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels. Biomaterials 27, 2265–2274.
Marelli, B., Ghezzi, C.E., James-Bhasin, M., Nazhat, S.N., 2015. Fabrication of injectable, cellular, anisotropic collagen tissue equivalents with modular fibrillar densities. Biomaterials 37, 183–193.
Matson, J.B., Stupp, S.I., 2012. Self-assembling peptide scaffolds for regenerative medicine. Chem. Commun. 48, 26–33.
Matyash, M., Despang, F., Ikonomidou, C., Gelinsky, M., 2014. Swelling and mechanical properties of alginate hydrogels with respect to promotion of neural growth. Tissue Eng. Part C Methods 20, 401–411.
McMurtrey, R.J., 2014. Patterned and functionalized nanofiber scaffolds in three-dimensional hydrogel constructs enhance neurite outgrowth and directional control. J. Neural Eng. 11 066009.
Mealy, J.E., Chung, J.J., Jeong, H.-H., Issadore, D., Lee, D., Atluri, P., Burdick, J.A., 2018. Injectable granular hydrogels with multifunctional properties for biomedical applications. Adv. Mater. 30 e1705912.
Medberry, C.J., Crapo, P.M., Siu, B.F., Carruthers, C.A., Wolf, M.T., Nagarkar, S.P., Agrawal, V., Jones, K.E., Kelly, J., Johnson, S.A., Velankar, S.S., Watkins, S.C., Modo, M., Badylak, S.F., 2013. Hydrogels derived from central nervous system extracellular matrix. Biomaterials 34, 1033–1040.
Mehrban, N., Zhu, B., Tamagnini, F., Young, F.I., Wasmuth, A., Hudson, K.L., Thomson, A.R., Birchall, M.A., Randall, A.D., Song, B., Woolfson, D.N., 2015. Functionalized α-helical peptide hydrogels for neural tissue engineering. ACS Biomater. Sci. Eng. 1, 431–439.
Mei, Y., Saha, K., Bogatyrev, S.R., Yang, J., Hook, A.L., Kalcioglu, Z.I., Cho, S.-W., Mitalipova, M., Pyzocha, N., Rojas, F., Van Vliet, K.J., Davies, M.C., Alexander, M.R., Langer, R., Jaenisch, R., Anderson, D.G., 2010. Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. Nat. Mater. 9, 768–778.
Meinhardt, A., Eberle, D., Tazaki, A., Ranga, A., Niesche, M., Wilsch-Bräuninger, M., Stec, A., Schackert, G., Lutolf, M., Tanaka, E.M., 2014. 3D reconstitution of the patterned neural tube from embryonic stem cells. Stem Cell Reports 3, 987–999.
Moriarty, N., Pandit, A., Dowd, E., 2017. Encapsulation of primary dopaminergic neurons in a GDNF-loaded collagen hydrogel increases their survival, re-innervation and function after intra-striatal transplantation. Sci. Rep. 7 16033.
Mredha, M.T.I., Guo, Y.Z., Nonoyama, T., Nakajima, T., Kurokawa, T., Gong, J.P., 2018. A facile method to fabricate anisotropic hydrogels with perfectly aligned hierarchical fibrous structures. Adv. Mater. 30. https://doi.org/10.1002/adma.201704937.
Nakajima, Y.-I., 2018. Mitotic spindle orientation in epithelial homeostasis and plasticity. J. Biochem. 164, 277–284.
Namba, R.M., Cole, A.A., Bjugstad, K.B., Mahoney, M.J., 2009. Development of porous PEG hydrogels that enable efficient, uniform cell-seeding and permit early neural process extension. Acta Biomater. 5, 1884–1897.
Navaei-Nigjeh, M., Amoabedini, G., Noroozi, A., Azami, M., Asmani, M.N., Ebrahimi-Barough, S., Saberi, H., Ai, A., Ai, J., 2014. Enhancing neuronal growth from human endometrial stem cells derived neuron-like cells in three-dimensional fibrin gel for nerve tissue engineering. J. Biomed. Mater. Res. A 102, 2533–2543.
Nih, L.R., Sideris, E., Carmichael, S.T., Segura, T., 2017. Injection of microporous annealing particle (MAP) hydrogels in the stroke cavity reduces gliosis and inflammation and promotes npc migration to the lesion. Adv. Mater. 29. https://doi.org/10.1002/adma.201606471.
Nii, M., Lai, J.H., Keeney, M., Han, L.-H., Behn, A., Imanbayev, G., Yang, F., 2013. The effects of interactive mechanical and biochemical niche signaling on osteogenic differentiation of adipose-derived stem cells using combinatorial hydrogels. Acta Biomater. 9, 5475–5483.
O'Rourke, C., Lee-Reeves, C., Drake, R.A., Cameron, G.W., Loughlin, A.J., Phillips, J.B., 2017. Adapting tissue-engineered in vitro CNS models for high-throughput study of neurodegeneration. J. Tissue Eng. 8 2041731417697920.
Omidinia-Anarkoli, A., Boesveld, S., Tuvshindorj, U., Rose, J.C., Haraszti, T., De Laporte, L., 2017. An injectable hybrid hydrogel with oriented short fibers induces unidirectional growth of functional nerve cells. Small 13. https://doi.org/10.1002/smll.201702207.
Ono, K., Saito, Y., Yura, H., Ishikawa, K., Kurita, A., Akaike, T., Ishihara, M., 2000. Photocrosslinkable chitosan as a biological adhesive. J. Biomed. Mater. Res. 49, 289–295.
Onoe, H., Kato-Negishi, M., Itou, A., Takeuchi, S., 2016. Differentiation induction of mouse neural stem cells in hydrogel tubular microenvironments with controlled tube dimensions. Adv. Healthc. Mater. 5, 1104–1111.
Palazzolo, G., Broguiere, N., Cenciarelli, O., Dermutz, H., Zenobi-Wong, M., 2015. Ultrasoft alginate hydrogels support long-term three-dimensional functional neuronal networks. Tissue Eng. Part A. https://doi.org/10.1089/ten.TEA.2014.0518.
Palu, E., Liesi, P., 2002. Differential distribution of laminins in Alzheimer disease and normal human brain tissue. J. Neurosci. Res. 69, 243–256.
Park, J., Lim, E., Back, S., Na, H., Park, Y., Sun, K., 2010. Nerve regeneration following spinal cord injury using matrix metalloproteinase-sensitive, hyaluronic acid-based biomimetic hydrogel scaffold containing brain-derived neurotrophic factor. J. Biomed. Mater. Res. A 93, 1091–1099.
Pashuck, E.T., Cui, H., Stupp, S.I., 2010. Tuning supramolecular rigidity of peptide fibers through molecular structure. J. Am. Chem. Soc. 132, 6041–6046.
Phillips, J.B., Bunting, S.C.J., Hall, S.M., Brown, R.A., 2005. Neural tissue engineering: a self-organizing collagen guidance conduit. Tissue Eng. 11, 1611–1617.
Piantino, J., Burdick, J.A., Goldberg, D., Langer, R., Benowitz, L.I., 2006. An injectable, biodegradable hydrogel for trophic factor delivery enhances axonal rewiring and improves performance after spinal cord injury. Exp. Neurol. 201, 359–367.
Ranga, A., Girgin, M., Meinhardt, A., Eberle, D., Caiazzo, M., Tanaka, E.M., Lutolf, M.P., 2016. Neural tube morphogenesis in synthetic 3D microenvironments. Proc. Natl. Acad. Sci. U. S. A. 113, E6831–E6839.
Ranga, A., Gobaa, S., Okawa, Y., Mosiewicz, K., Negro, A., Lutolf, M.P., 2014. 3D niche microarrays for systems-level analyses of cell fate. Nat. Commun. 5, 4324.
Rickett, T.A., Amoozgar, Z., Tuchek, C.A., Park, J., Yeo, Y., Shi, R., 2011. Rapidly photo-cross-linkable chitosan hydrogel for peripheral neurosurgeries. Biomacromolecules 12, 57–65.
Roach, P., Kose Dunn, M., Fricker, R., 2017. Tissue engineered organoids for neural network modelling. ATROA 3. https://doi.org/10.15406/atroa.2017.03.00066.
Roam, J.L., Yan, Y., Nguyen, P.K., Kinstlinger, I.S., Leuchter, M.K., Hunter, D.A., Wood, M.D., Elbert, D.L., 2015. A modular, plasmin-sensitive, clickable poly(ethylene glycol)-heparin-laminin microsphere system for establishing growth factor gradients

in nerve guidance conduits. Biomaterials 72, 112–124.
Rocca, M., Fragasso, A., Liu, W., Heinrich, M.A., Zhang, Y.S., 2018. Embedded Multimaterial Extrusion Bioprinting. SLAS Technol. 23, 154–163.
Rose, J.C., Cámara-Torres, M., Rahimi, K., Köhler, J., Möller, M., De Laporte, L., 2017. Nerve cells decide to orient inside an injectable hydrogel with minimal structural guidance. Nano Lett. 17, 3782–3791.
Royce Hynes, S., McGregor, L.M., Ford Rauch, M., Lavik, E.B., 2007. Photopolymerized poly(ethylene glycol)/poly(L-lysine) hydrogels for the delivery of neural progenitor cells. J. Biomater. Sci. Polym. Ed. 18, 1017–1030.
Ryu, J.K., Davalos, D., Akassoglou, K., 2009. Fibrinogen signal transduction in the nervous system. J. Thromb. Haemost. 7 (Suppl. 1), 151–154.
Sabnis, A., Rahimi, M., Chapman, C., Nguyen, K.T., 2009. Cytocompatibility studies of an in situ photopolymerized thermoresponsive hydrogel nanoparticle system using human aortic smooth muscle cells. J. Biomed. Mater. Res. A 91, 52–59.
Sachs, P.C., Mollica, P.A., Bruno, R.D., 2017. Tissue specific microenvironments: a key tool for tissue engineering and regenerative medicine. J. Biol. Eng. 11, 34.
Saha, K., Keung, A.J., Irwin, E.F., Li, Y., Little, L., Schaffer, D.V., Healy, K.E., 2008. Substrate modulus directs neural stem cell behavior. Biophys. J. 95, 4426–4438.
Saldin, L.T., Cramer, M.C., Velankar, S.S., White, L.J., Badylak, S.F., 2017. Extracellular matrix hydrogels from decellularized tissues: structure and function. Acta Biomater. 49, 1–15.
Sandvig, I., Karstensen, K., Rokstad, A.M., Aachmann, F.L., Formo, K., Sandvig, A., Skjåk-Bræk, G., Strand, B.L., 2015. RGD-peptide modified alginate by a chemoenzymatic strategy for tissue engineering applications. J. Biomed. Mater. Res. A 103, 896–906.
Sano, K., Ishida, Y., Aida, T., 2018. Synthesis of anisotropic hydrogels and their applications. Angew. Chem. Int. Ed. Eng. 57, 2532–2543.
Sant, S., Hancock, M.J., Donnelly, J.P., Iyer, D., Khademhosseini, A., 2010. Biomimetic gradient hydrogels for tissue engineering. Can. J. Chem. Eng. 88, 899–911.
Scadden, D.T., 2014. Nice neighborhood: emerging concepts of the stem cell niche. Cell 157, 41–50.
Schaefer, M.H., Yang, J.-S., Serrano, L., Kiel, C., 2014. Protein conservation and variation suggest mechanisms of cell type-specific modulation of signaling pathways. PLoS Comput. Biol. 10 e1003659.
Schuh, C.M.A.P., Morton, T.J., Banerjee, A., Grasl, C., Schima, H., Schmidhammer, R., Redl, H., Ruenzler, D., 2014. Activated Schwann cell-like cells on aligned fibrin-poly (lactic-co-glycolic acid) structures: a novel construct for application in peripheral nerve regeneration. Cells Tissues Organs 200, 287–299.
Scott, J.B., Afshari, M., Kotek, R., Saul, J.M., 2011a. The promotion of axon extension in vitro using polymer-templated fibrin scaffolds. Biomaterials 32, 4830–4839.
Scott, R.A., Elbert, D.L., Willits, R.K., 2011b. Modular poly(ethylene glycol) scaffolds provide the ability to decouple the effects of stiffness and protein concentration on PC12 cells. Acta Biomater. 7, 3841–3849.
Segura, T., Anderson, B.C., Chung, P.H., Webber, R.E., Shull, K.R., Shea, L.D., 2005. Crosslinked hyaluronic acid hydrogels: a strategy to functionalize and pattern. Biomaterials 26, 359–371.
Seidlits, S.K., Khaing, Z.Z., Petersen, R.R., Nickels, J.D., Vanscoy, J.E., Shear, J.B., Schmidt, C.E., 2010. The effects of hyaluronic acid hydrogels with tunable mechanical properties on neural progenitor cell differentiation. Biomaterials 31, 3930–3940.
Sellers, D.L., Kim, T.H., Mount, C.W., Pun, S.H., Horner, P.J., 2014. Poly(lactic-co-glycolic) acid microspheres encapsulated in Pluronic F-127 prolong hirudin delivery and improve functional recovery from a demyelination lesion. Biomaterials 35, 8895–8902.
Silva, N.A., Cooke, M.J., Tam, R.Y., Sousa, N., Salgado, A.J., Reis, R.L., Shoichet, M.S., 2012. The effects of peptide modified gellan gum and olfactory ensheathing glia cells on neural stem/progenitor cell fate. Biomaterials 33, 6345–6354.
Skop, N.B., Calderon, F., Cho, C.H., Gandhi, C.D., Levison, S.W., 2016. Optimizing a multifunctional microsphere scaffold to improve neural precursor cell transplantation for traumatic brain injury repair. J. Tissue Eng. Regen. Med. 10, E419–E432.
Smith, A.W., Segar, C.E., Nguyen, P.K., MacEwan, M.R., Efimov, I.R., Elbert, D.L., 2012. Long-term culture of HL-1 cardiomyocytes in modular poly(ethylene glycol) microsphere-based scaffolds crosslinked in the phase-separated state. Acta Biomater. 8, 31–40.
Song, G., Zhang, L., He, C., Fang, D.-C., Whitten, P.G., Wang, H., 2013. Facile fabrication of tough hydrogels physically cross-linked by strong cooperative hydrogen bonding. Macromolecules 46, 7423–7435.
Song, Y., Li, Y., Zheng, Q., Wu, K., Guo, X., Wu, Y., Yin, M., Wu, Q., Fu, X., 2011. Neural progenitor cells survival and neuronal differentiation in peptide-based hydrogels. J. Biomater. Sci. Polym. Ed. 22, 475–487.
Stabenfeldt, S.E., LaPlaca, M.C., 2011. Variations in rigidity and ligand density influence neuronal response in methylcellulose-laminin hydrogels. Acta Biomater. 7, 4102–4108.
Stanwick, J.C., Baumann, M.D., Shoichet, M.S., 2012. Enhanced neurotrophin-3 bioactivity and release from a nanoparticle-loaded composite hydrogel. J. Control. Release 160, 666–675.
Stenekes, R.J., Talsma, H., Hennink, W.E., 2001. Formation of dextran hydrogels by crystallization. Biomaterials 22, 1891–1898.
Strappe, P.M., Hampton, D.W., Cachon-Gonzalez, B., Fawcett, J.W., Lever, A., 2005. Delivery of a lentiviral vector in a Pluronic F127 gel to cells of the central nervous system. Eur. J. Pharm. Biopharm. 61, 126–133.
Struzyna, L.A., Wolf, J.A., Mietus, C.J., Adewole, D.O., Chen, H.I., Smith, D.H., Cullen, D.K., 2015. Rebuilding brain circuitry with living micro-tissue engineered neural networks. Tissue Eng. Part A 21, 2744–2756.
Sun, W., Incitti, T., Migliaresi, C., Quattrone, A., Casarosa, S., Motta, A., 2017. Viability and neuronal differentiation of neural stem cells encapsulated in silk fibroin hydrogel functionalized with an IKVAV peptide. J. Tissue Eng. Regen. Med. 11, 1532–1541.
Swindle-Reilly, K.E., Papke, J.B., Kutosky, H.P., Throm, A., Hammer, J.A., Harkins, A.B., Willits, R.K., 2012. The impact of laminin on 3D neurite extension in collagen gels. J. Neural Eng. 9 046007.
Tam, R.Y., Fuehrmann, T., Mitrousis, N., Shoichet, M.S., 2014. Regenerative therapies for central nervous system diseases: a biomaterials approach. Neuropsychopharmacology 39, 169–188.
Tang-Schomer, M.D., White, J.D., Tien, L.W., Schmitt, L.I., Valentin, T.M., Graziano, D.J., Hopkins, A.M., Omenetto, F.G., Haydon, P.G., Kaplan, D.L., 2014. Bioengineered functional brain-like cortical tissue. Proc. Natl. Acad. Sci. U. S. A. 111, 13811–13816.
Tate, M.C., Shear, D.A., Hoffman, S.W., Stein, D.G., LaPlaca, M.C., 2001. Biocompatibility of methylcellulose-based constructs designed for intracerebral gelation following experimental traumatic brain injury. Biomaterials 22, 1113–1123.
Tavakol, S., Aligholi, H., Gorji, A., Eshaghabadi, A., Hoveizi, E., Tavakol, B., Rezayat, S.M., Ai, J., 2014. Thermogel nanofiber induces human endometrial-derived stromal cells to neural differentiation: in vitro and in vivo studies in rat. J. Biomed. Mater. Res. 102, 4590–4597.
Taylor, S.J., McDonald, J.W., Sakiyama-Elbert, S.E., 2004. Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. J. Control. Release 98, 281–294.
Taylor, S.J., Rosenzweig, E.S., McDonald, J.W., Sakiyama-Elbert, S.E., 2006. Delivery of neurotrophin-3 from fibrin enhances neuronal fiber sprouting after spinal cord injury. J. Control. Release 113, 226–235.
Tedesco, M.T., Di Lisa, D., Massobrio, P., Colistra, N., Pesce, M., Catelani, T., Dellacasa, E., Raiteri, R., Martinoia, S., Pastorino, L., 2018. Soft chitosan microbeads scaffold for 3D functional neuronal networks. Biomaterials 156, 159–171.
Teixeira, L.S.M., Feijen, J., van Blitterswijk, C.A., Dijkstra, P.J., Karperien, M., 2012. Enzyme-catalyzed crosslinkable hydrogels: emerging strategies for tissue engineering. Biomaterials 33, 1281–1290.
Tomaselli, K.J., Neugebauer, K.M., Bixby, J.L., Lilien, J., Reichardt, L.F., 1988. N-cadherin and integrins: two receptor systems that mediate neuronal process outgrowth on astrocyte surfaces. Neuron 1, 33–43.
Tsai, E.C., Dalton, P.D., Shoichet, M.S., Tator, C.H., 2006. Matrix inclusion within synthetic hydrogel guidance channels improves specific supraspinal and local axonal regeneration after complete spinal cord transection. Biomaterials 27, 519–533.
Tseng, T.-C., Tao, L., Hsieh, F.-Y., Wei, Y., Chiu, I.-M., Hsu, S.-H., 2015. An injectable, self-healing hydrogel to repair the central nervous system. Adv. Mater. 27, 3518–3524.
Tunesi, M., Batelli, S., Rodilossi, S., Russo, T., Grimaldi, A., Forloni, G., Ambrosio, L., Cigada, A., Gloria, A., Albani, D., Giordano, C., 2013. Development and analysis of semi-interpenetrating polymer networks for brain injection in neurodegenerative disorders. Int. J. Artif. Organs 36, 762–774.
Turunen, S., Haaparanta, A.-M., Äänismaa, R., Kellomäki, M., 2013. Chemical and topographical patterning of hydrogels for neural cell guidance in vitro. J. Tissue Eng. Regen. Med. 7, 253–270.
Uemura, M., Refaat, M.M., Shinoyama, M., Hayashi, H., Hashimoto, N., Takahashi, J., 2010. Matrigel supports survival and neuronal differentiation of grafted embryonic stem cell-derived neural precursor cells. J. Neurosci. Res. 88, 542–551.
Ulijn, R.V., Bibi, N., Jayawarna, V., Thornton, P.D., Todd, S.J., Mart, R.J., Smith, A.M., Gough, J.E., 2007. Bioresponsive hydrogels. Mater. Today 10, 40–48.
Uzel, S.G.M., Amadi, O.C., Pearl, T.M., Lee, R.T., So, P.T.C., Kamm, R.D., 2016. Simultaneous or sequential orthogonal gradient formation in a 3d cell culture microfluidic platform. Small 12, 612–622.
Valmikinathan, C.M., Mukhatyar, V.J., Jain, A., Karumbaiah, L., Dasari, M., Bellamkonda, R.V., 2012. Photocrosslinkable chitosan based hydrogels for neural tissue engineering. Soft Matter 8, 1964–1976.
Vega L, J.C.M., Lee, M.K., Qin, E.C., Rich, M., Lee, K.Y., Kim, D.H., Chung, H.J., Leckband, D.E., Kong, H., 2016. Three dimensional conjugation of recombinant N-cadherin to a hydrogel for *in vitro* anisotropic neural growth. J. Mater. Chem. B Mater. Biol. Med. 4, 6803–6811.
Wang, C., Yue, H., Feng, Q., Xu, B., Bian, L., Shi, P., 2018. Injectable nano-reinforced shape-memory hydrogel system for regenerating spinal cord tissue from traumatic injury. ACS Appl. Mater. Interfaces. https://doi.org/10.1021/acsami.8b08929.
Wang, G., Wang, X., Huang, L., 2017. Feasibility of chitosan-alginate (Chi-Alg) hydrogel used as scaffold for neural tissue engineering: a pilot study in vitro. Biotechnol. Biotechnol. Equip. 31, 766–773.
Wang, H., Heilshorn, S.C., 2015. Adaptable hydrogel networks with reversible linkages for tissue engineering. Adv. Mater. 27, 3717–3736.
Wang, L.-S., Chung, J.E., Chan, P.P.-Y., Kurisawa, M., 2010. Injectable biodegradable hydrogels with tunable mechanical properties for the stimulation of neurogenesic differentiation of human mesenchymal stem cells in 3D culture. Biomaterials 31, 1148–1157.
Wang, T., Turhan, M., Gunasekaran, S., 2004. Selected properties of pH-sensitive, biodegradable chitosan–poly(vinyl alcohol) hydrogel. Polym. Int. 53, 911–918.
Weber, L.M., Lopez, C.G., Anseth, K.S., 2009. Effects of PEG hydrogel crosslinking density on protein diffusion and encapsulated islet survival and function. J. Biomed. Mater. Res. A 90, 720–729.
Wei, Z., Zhao, J., Chen, Y.M., Zhang, P., Zhang, Q., 2016. Self-healing polysaccharide-based hydrogels as injectable carriers for neural stem cells. Sci. Rep. 6 37841.
Williams, C.A., Lavik, E.B., 2009. Engineering the CNS stem cell microenvironment. Regen. Med. 4, 865–877.
Wozniak, M.A., Modzelewska, K., Kwong, L., Keely, P.J., 2004. Focal adhesion regulation of cell behavior. Biochim. Biophys. Acta 1692, 103–119.
Wu, H.-F., Cen, J.-S., Zhong, Q., Chen, L., Wang, J., Deng, D.Y.B., Wan, Y., 2013. The promotion of functional recovery and nerve regeneration after spinal cord injury by lentiviral vectors encoding Lingo-1 shRNA delivered by Pluronic F-127. Biomaterials 34, 1686–1700.
Xu, H.-L., Tian, F.-R., Lu, C.-T., Xu, J., Fan, Z.-L., Yang, J.-J., Chen, P.-P., Huang, Y.-D.,

ARTICLE IN PRESS

Xiao, J., Zhao, Y.-Z., 2016. Thermo-sensitive hydrogels combined with decellularised matrix deliver bFGF for the functional recovery of rats after a spinal cord injury. Sci. Rep. 6 38332.

Yang, Y.-H., Khan, Z., Ma, C., Lim, H.J., Smith Callahan, L.A., 2015. Optimization of adhesive conditions for neural differentiation of murine embryonic stem cells using hydrogels functionalized with continuous Ile-Lys-Val-Ala-Val concentration gradients. Acta Biomater. https://doi.org/10.1016/j.actbio.2015.04.031.

Yao, L., Damodaran, G., Nikolskaya, N., Gorman, A.M., Windebank, A., Pandit, A., 2010. The effect of laminin peptide gradient in enzymatically cross-linked collagen scaffolds on neurite growth. J. Biomed. Mater. Res. A 92, 484–492.

Yu, T.T., Shoichet, M.S., 2005. Guided cell adhesion and outgrowth in peptide-modified channels for neural tissue engineering. Biomaterials 26, 1507–1514.

Yuan, J.-J., Jin, R.-H., 2005. Fibrous crystalline hydrogels formed from polymers possessing a linear poly(ethyleneimine) backbone. Langmuir 21, 3136–3145.

Yung, C.W., Wu, L.Q., Tullman, J.A., Payne, G.F., Bentley, W.E., Barbari, T.A., 2007. Transglutaminase crosslinked gelatin as a tissue engineering scaffold. J. Biomed. Mater. Res. Part A 83, 1039–1046.

Zhang, G., Chen, Y., Deng, Y., Ngai, T., Wang, C., 2017. Dynamic supramolecular hydrogels: regulating hydrogel properties through self-complementary quadruple hydrogen bonds and thermo-switch. ACS Macro Lett. 6, 641–646.

Zhang, Z.-N., Freitas, B.C., Qian, H., Lux, J., Acab, A., Trujillo, C.A., Herai, R.H., Nguyen Huu, V.A., Wen, J.H., Joshi-Barr, S., Karpiak, J.V., Engler, A.J., Fu, X.-D., Muotri, A.R., Almutairi, A., 2016. Layered hydrogels accelerate iPSC-derived neuronal maturation and reveal migration defects caused by MeCP2 dysfunction. Proc. Natl. Acad. Sci. U. S. A. 113, 3185–3190.

Zhao, T., Sellers, D.L., Cheng, Y., Horner, P.J., Pun, S.H., 2017a. Tunable, injectable hydrogels based on peptide-cross-linked, cyclized polymer nanoparticles for neural progenitor cell delivery. Biomacromolecules 18, 2723–2731.

Zhao, Z., Fang, R., Rong, Q., Liu, M., 2017b. Bioinspired nanocomposite hydrogels with highly ordered structures. Adv. Mater. 29. https://doi.org/10.1002/adma.201703045.

Zhou, W., Stukel, J.M., Cebull, H.L., Willits, R.K., 2016. Tuning the mechanical properties of poly(ethylene glycol) microgel-based scaffolds to increase 3D Schwann cell proliferation. Macromol. Biosci. 16, 535–544.

Zhou, Z., Yu, P., Geller, H.M., Ober, C.K., 2012. The role of hydrogels with tethered acetylcholine functionality on the adhesion and viability of hippocampal neurons and glial cells. Biomaterials 33, 2473–2481.

Zuidema, J.M., Pap, M.M., Jaroch, D.B., Morrison, F.A., Gilbert, R.J., 2011. Fabrication and characterization of tunable polysaccharide hydrogel blends for neural repair. Acta Biomater. 7, 1634–1643.