Acta Pharmaceutica Sinica B 2018;8(5):756–766

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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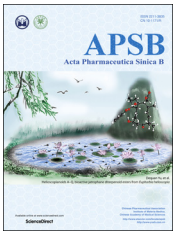

REVIEW

3D tissue engineering, an emerging technique for pharmaceutical research

Gregory Jensen, Christian Morrill, Yu Huang*

Department of Biological Engineering, Utah State University, Logan, UT, 84322, USA

Received 20 January 2018; received in revised form 9 March 2018; accepted 13 March 2018

KEY WORDS

Tissue engineering;
Decellularization;
Neurodegenerative disease;
Drug screening;
Neuroinflammation;
3D cell culture

Abstract Tissue engineering and the tissue engineering model have shown promise in improving methods of drug delivery, drug action, and drug discovery in pharmaceutical research for the attenuation of the central nervous system inflammatory response. Such inflammation contributes to the lack of regenerative ability of neural cells, as well as the temporary and permanent loss of function associated with neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury. This review is focused specifically on the recent advances in the tissue engineering model made by altering scaffold biophysical and biochemical properties for use in the treatment of neurodegenerative diseases. A portion of this article will also be spent on the review of recent progress made in extracellular matrix decellularization as a new and innovative scaffold for disease treatment.

*Corresponding author.
E-mail address: yu.huang@usu.edu (Yu Huang).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2018.03.006

1. Introduction

1.1. Background

Neurodegenerative diseases and brain disorders have been some of the targets for pharmaceutical research. There are an estimated 5.4 million cases of Alzheimer's disease and 1 million cases of Parkinson's disease in the US as of 2016. It is projected that the number of individuals suffering from Alzheimer's will increase to 16 million by 2050, while approximately 60,000 new diagnoses of Parkinson's occur every year in the US alone[1,2]. The substantial increase in the number of occurrences of these and other neurodegenerative diseases will cost billions of dollars and require countless hours of personal medical care worldwide. Due to the tremendous burden to countries, families, and individuals brought on by these diseases, an emphasis has been placed on improving current therapies and finding novel remedies to treat these disorders.

One challenge associated with the treatment of neurodegenerative diseases is the attenuation of neuroinflammation brought on by these disorders. This inflammatory response is thought to be a main driving force behind the progression of these diseases, which contributes to the loss of neural cells and lack of functional recovery after disease onset[3]. Promising results in the treatment of many neurodegenerative diseases have been obtained during preliminary trials by mitigating neural inflammation affiliated with these diseases[4–6]. Because of these findings, one area of focus is to explore the use of known and novel anti-inflammatory compounds for the treatment of neurodegenerative disorders, in hopes of finding more effective therapies for individuals suffering from these ailments. Even though anti-inflammatory compounds are promising therapies, pharmaceutical research still needs to find more feasible methods to screen for potential anti-inflammatory agents as well as to find more effective ways to deliver these drugs to sites of neuroinflammation and degeneration. Advancements made in 3-dimensional (3D) tissue mimics, by employing tissue engineering principles, have improved preclinical drug screening trials[7,8]; the results of which have provided unique platforms for the enhancement of drug delivery to areas of need[9–12].

This article reviews recent progress in pharmaceutical science made specifically by application of the tissue engineering model and 3D tissue mimics. Utilization of this model in the advancement of drug screening procedures and drug delivery methods will be thoroughly reviewed as a novel approach to benefit the treatment of neurological diseases. A novel decellularization technique will also be summarized as a 3D culture material that mimics the natural ECM of the brain. This new approach will further open the door to continued progress in solving the neurodegenerative disease crisis by providing more reliable drug screening and toxicity results from preclinical trials. In this way, the use of tissue engineering principles will enhance pharmaceutical and neurodegenerative disease research.

1.2. Role of microglia in neurodegenerative diseases

Immune systems have become targets of emerging pharmaceutical research, including that of the central nervous system (CNS). Unlike macrophages that serve as the immune cells for the rest of the body, microgliaare the resident immune cells uniquely of the CNS and thus have many important functions in the brain. Unregulated and prolonged activation of these immune cells may contribute to the self-propelling nature of neurodegenerative diseases[3,13–16].

Resting, or inactivated microglia, are dynamic cells that constantly survey their surroundings by extending and contracting processes protruding from their cell body. These processes are able to detect minute change in their environment and in this way are able to identify and respond to signals or foreign objects that require an immune response[17]. In an attempt to remove harmful substances, this immune response is often associated with the release of many pro-inflammatory mediators that are modulated by activated microglia, such as superoxide, nitric oxide, tumor necrosis factor-alpha (TNF-α), and inflammatory prostaglandins that induce inflammation in the central nervous system[16]. The purpose of these pro-inflammatory mediators is to rid the central nervous system of invading pathogens or foreign objects, but these mediators are also toxic to neural cells and can promote neural cell damage and death.

Upon damage or death of neural cells, soluble neuron-injury factors/cytokines such as μ-calpain, MMP3, α-synuclein, and neuromelanin are often released from neural cells and are received by receptors on the surface of microglial cells. These soluble neuron-injury factors activate more microglial cells or serve to prolong the activated state of previously activated microglial cells. These activated microglial cells continue to release pro-inflammatory mediators that further damage neural cells that, in turn, continue to activate microglial cells. This cycle, termed reactive microgliosis, chronically activates the microglial cell inflammatory response and self-propels neurotoxicity (Fig. 1).

This heightened inflammatory response and state of reactive microgliosis has been commonly observed in neurodegenerative diseases such as Parkinson's disease[3], Alzheimer's disease[14], amyotrophic lateral sclerosis[18], and traumatic brain injury[15,19]. Due to the progressive nature of these and other neurodegenerative diseases, reactive microgliosis has been suspected as a main contributor to the progression and lack of functional recovery associated with neurodegenerative diseases. A consequence of reactive microgliosis is that an emphasis has been placed on screening for compounds that exhibit anti-inflammatory effects for the treatment of neurodegenerative diseases.

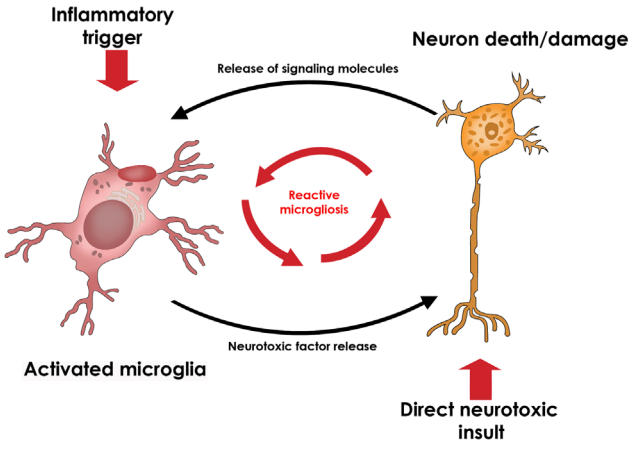

Figure 1 Reactive microgliosis perpetuates neuron damage and inflammation. Stimulation of microglia with pro-inflammatory triggers, as well as direct neuron damage, results in microglial activation and the release of neurotoxic factors. After damaged by a pro-inflammatory trigger or direct neurotoxin, the neuron releases microglial activators, which propagates the cycle.

1.3. *The tissue engineering model*

Tissue engineering is the application of engineering principles to treat, or replace, damaged tissues and organs. The tissue engineering model employs the use of 3D matrices to culture cells, and to produce living tissues, which mimic the morphology and function of what naturally occurs *in vivo*. These tissue mimics can be exploited for studies of disease propagation and progression, drug discovery and compound screening, and even tissue repair and replacement. The use of organoids, 3D scaffolds, and decellularization techniques are viable ways to create 3D materials for use in tissue specific research.

One common method for producing tissue mimics is the use of 3D organoids. An organoid is defined as "an *in vitro* 3D cellular cluster derived exclusively from primary tissue, embryonic stem cells, or induced pluripotent stem cells (iPSCs), capable of self-renewal and self-organization, while exhibiting similar organ functionality as the tissue of origin"[20] ("Cell Culture"). An advantage of the organoid model is the ability to study disease development and progression due to the ability of organoids to mimic the morphology and functionality of the tissues which they are derived from. A study by Lancaster et al.[21] took advantage of the organoid model to better determine the cause of hypoplasia in patients suffering from microcephaly. This study showed that non-functional CDK5RAP2 genes were a likely cause of premature neural differentiation, resulting in hypoplasia of the organoid cells[21]. Another benefit of this model is the potential for developing more personalized medicine by culturing organoids from healthy and diseased tissues of patients. By using the organoid system, it is possible to screen for potential compounds that will affect the diseased tissue with minimal side effects to healthy tissues[20]. The organoid model is also being used for the study of the Zika virus and its effects on human brain development[22,23].

A second approach for the creation of tissue mimics is to seed cells onto a 3D matrix, or to disperse cells into a liquid hydrogel which will form into a 3D scaffold during the polymerization process[24] (Fig. 2 "Engineered Materials"). These scaffolds are formed from synthetic, or natural, polymers and the chemical and physical properties of the scaffold, such as porosity, stiffness, and incorporation of bioactive molecules, can easily be altered to fit the needs of the seeded cells. The benefit of this method is the ability to customize the microenvironment in which cells are cultured. Recently, advances in 3D printing have made the creation of printed matrices for tissue engineering possible. Specifically, 3D printed scaffolds are under investigation for use in bone tissue engineering purposes[25], but may have applications for softer tissues.

A novel alternative to conventional culture techniques is to use a decellularization method to obtain material for a 3D matrix. This method involves removal of cells from native tissues or organs, while maintaining extracellular matrix (ECM) integrity and keeping important bioactive molecules intact[26,27] (Fig. 2 "Animal-Based Materials"). This method provides a 3D microenvironment for seeded cells with most of the necessary chemical cues intact for healthy growth and development.

When applying tissue engineering principles to neural tissues, the mechanical properties of the brain must be considered when determining an appropriate method to use. Brain tissue is a soft tissue with an elastic modulus in the hundreds of pascals range, while other tissues in the human body have much higher elastic moduli[28,29]. It has been shown that cells respond to the resistance, or stiffness, of the substrate on which they are cultured[30], with the substrate stiffness directly affecting the proliferation and differentiation of stem cells to neural cells[29].

As opposed to other tissues in the body, the primary immune cells of the brain are microglial cells. Microglial cells are beneficial, but have been are known to produce pro-inflammatory molecules that may contribute to disease progression[33]. Acceptable tissue engineering models of the human brain should include microglial cells to more accurately reflect inflammatory pathways in the brain.

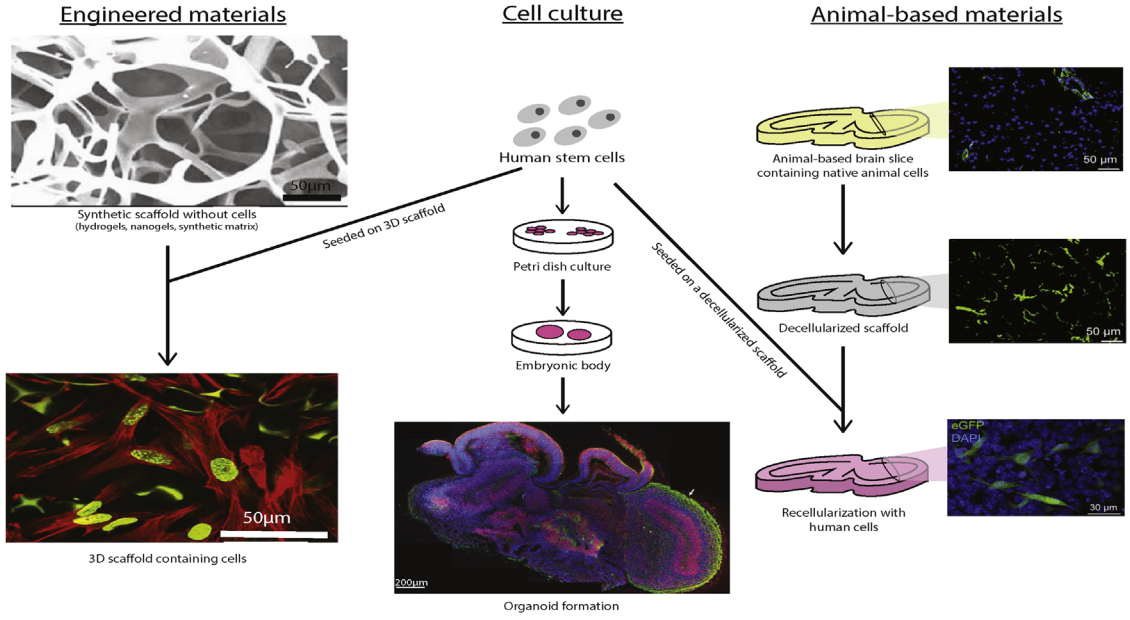

Figure 2 The tissue engineering model and different ways 3D microenvironments are formed. Gray scaffolds represent the absence of cells, pink scaffolds represent the presence of seeded human stem cells, and the yellow scaffold represents the presence of animal cells before tissue decellularization. Microscopic images in the engineered materials section, cell culture section, and animal-based materials section were adapted with permission from Mandal et al.[31], Lancaster et al.[21], and De Waele et al.[32], respectively.

2. Drug candidates for neurodegenerative disease

2.1. *Anti-inflammatory drug candidates*

Many compounds and bioactive molecules have shown activity in *in vitro* and in animal models against neuroinflammation and reactive microgliosis. Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of compounds that are well known to have anti-inflammatory, analgesic, and antipyretic effects. As such, they show great promise in the treatment of inflammation associated with neurodegenerative diseases. It is well known that NSAIDs help to regulate inflammation in the body by inhibiting the activity of cyclooxygenase 1 (COX 1), cyclooxygenase 2 (COX 2), and prostaglandin (PG) biosynthetic enzymes[34,35]. PGs are mediators of inflammation and they are synthesized *in vivo* from arachidonic acid by the action of COX enzymes.

Of particular interest in the central nervous system is the synthesis of prostaglandin E_2 (PGE_2), which is the major prostaglandin associated with the presence of inflammation in the brain and spinal cord[36]. Several NSAIDs have shown promise for the attenuation of the inflammatory response by reducing the levels of PGE_2 in circulation, in animal models, once an insult to the brain has been experienced[4] (Table 1[5,37–47]). As prostaglandins are mediators of inflammation in the brain, the effective inhibition of their synthesis and release helps to reduce inflammation brought on by activated microglia. These anti-inflammatory effects have been displayed in disease models of Alzheimer's disease[48] and Parkinson's disease[49], and may be applied to other neurodegenerative diseases as well.

Other studies have been conducted to determine the effectiveness of natural compounds in the treatment of neuroinflammation. Although little is known about their mechanisms, many natural compounds show great promise in the treatment of neuroinflammation (Table 1). One study experimented with the anti-inflammatory effects of torilin, a compound isolated from the stem and root bark of *Ulmus davidiana* var. *japonica*, and determined that it was effective in reducing iNOS levels, COX 2 levels, and IL-1β levels in murine BV2 microglial cells after exposure to lipopolysaccharides (LPS)[40]. Another study focused on the anti-inflammatory effects of macelignan, a compound isolated from *Myristica fragrans* Houtt, on primary culture of rat microglial cells after exposure to LPS. This study showed that macelignan effectively reduced the concentrations of three known pro-inflammatory molecules: interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and nitrite (NO) in rat microglial cultures after exposure to LPS *in vitro*[41]. A thorough review by Pangestuti and Kim[6] demonstrated that many different species of marine algae have shown neuroprotective effects *in vivo* and *in vitro* by acting as antioxidants, by reducing neuroinflammation, and by inhibiting neuronal cell death[6,42,44]. Eicosanoyl-5-hydroxytryptamide (EHT), a component in coffee, also exhibited anti-inflammatory and neuroprotective effects in MPTP models of Parkinson's disease in mice after exposure to LPS[45]. Additionally, multiple compounds isolated from the root bar of *Lycium chinense* have shown to suppress the production of NO in LPS-induced BV2 cells[46]. In these ways, many natural compounds show promise in the treatment of neuroinflammation and neurodegenerative diseases.

Plasmalogens have also shown promise in the treatment of inflammation in neurodegenerative diseases (Table 1), especially Alzheimer's disease. Plasmalogens are glycerophospholipids that have a vinyl ether moiety on the first carbon of the glycerol backbone and are known to play important roles in membrane fluidity and cellular processes. A study was performed by Ifuku et al.[5] to determine the effect of plasmalogens on LPS-induced microglial activation in adult mice brains. As expected, LPS injections in the prefrontal cortex (PFC) of adult mice significantly increased the number of activated microglial cells as well as the amount of pro-inflammatory mediators such as IL-1β, TNF-α, reactive oxygen species, and reactive nitrogen species. It has been seen that administration of plasmalogens after LPS injection in the PFC of mice attenuated the microglial activation to control levels of mice that did not receive LPS injections. A side effect of the attenuated microglial activation was a significant decrease in the concentration of pro-inflammatory mediators back to control levels[5]. Due to the vinyl ether moiety located on the first carbon of the glycerol backbone, it is thought that plasmalogens have antioxidant effects[50,51] that may contribute to their anti-inflammatory properties by regulating free radical concentrations in the brain.

Table 1 Effect of different anti-inflammatory compounds on pro-inflammatory mediators in the CNS.

Drug	Effect	Status
Indomethacin (NSAID)	Reduced the PGE_2 level by 50% in rats (1 nmol/L)[37,38]	Approved drug
Piroxicam (NSAID)	Reduced the PGE_2 level by 50% (0.1 μmol/L[37,38]	Approved drug
Flurbiprofen (NSAID)	Reduced the PGE_2 level by 50% (0.1 μmol/L)[38,39]	Approved drug
Paracetamol (NSAID)	Reduced the PGE_2 level by 50% (7.6 μmol/L)[38]	Approved drug
Acetylsalicylic acid (NSAID)	Reduced the PGE_2 level by 50% (10 μmol/L) [38]	Approved drug
NS-398 (NSAID)	COX-2 inhibitor: reduced the PGE_2 levels by 50% (1–5 nmol/L)[38]	Chemical approved for research
Torillin	Reduced neurotoxic factors after LPS exposure in murine BV2 microglial cells[40]	Natural product/drug candidate
Macelignan	Reduced neurotoxic factors after LPS exposure in rat microglial cultures[41]	Natural product/drug candidate
Marine algae	Reduced pro-inflammatory mediators in murine BV2 and HT22 cell lines[42–44]	Natural product/drug candidate
EHT	Reduced pro-inflammatory mediators in cultured primary microglia after LPS exposure[45]	Natural product/drug candidate
Lycium chinense	Suppress production of NO in BV2 cells[46]	Natural product/drug candidate
Plasmalogens	Attenuation of microglia activation and of pro-inflammatory mediators in mice PFC[5]	Natural product/drug candidate
PHPB	Reduce levels of pro-inflammatory intermediates in mice[47]	Phase II clinical trials

A novel neuroprotective compound, potassium 2-(1-hydroxypentyl)-benzoate (PHPB), was shown to reduce levels of pro-inflammatory intermediates after LPS treatment in mice, and has shown potential in the treatment of Alzheimer's disease and ischemic stroke[47]. PHPB is currently in phase II clinical trial for ischemic stroke in China[47].

Even though many compounds show potential for treatment of neurodegenerative diseases in initial experiments, suitable models must still be developed to further explore a compound's effect on disease progression and native tissues before it can proceed to expensive clinical trials.

2.2. *3D tissue models as drug screening tools*

A specific challenge facing pharmaceutical companies and drug discovery is the high attrition rate of potential therapeutic compounds as they go from the *in vitro* to *in vivo* stage of drug research and development. It is estimated that less than 8% of drugs that enter phase I clinical trials will make it to market and this high attrition rate is made worse when factoring in that average costs to complete clinical trials range from 0.8 to 1.7 billion dollars[8,52,53]. Due to the high monetary cost of getting a new drug approved for use by the FDA, it is necessary to screen and dismiss compounds that are potentially ineffective or toxic as early in the compound evaluation process as possible[54]; often carried out through *in vitro* cell culture models. As a result, during the early stage, it is crucial to utilize models as close as possible to the *in vivo* counterpart.

Traditionally, 2-dimensional (2D) cell cultures have been used as an initial means to determine a compound's potential use as a novel drug. Although this 2D method is convenient, research has shown that the use of a 2D cell culture has potentially significant drawbacks producing misleading toxicological data[55]. It has been seen experimentally that cells grown in 2D cultures exhibit different morphologies, polarity, receptor expression, extra-cellular matrix interaction, cell–cell interactions, and other chemical and physical properties, when compared to what is observed *in vivo*[54,56]. These differences in cell structure and behavior in 2D cultures have been attributed to the failure of numerous compounds during *in vivo* experiments that showed promise in preclinical trials. However, 3D cell culturing techniques, used often in tissue engineering applications, have shown much stronger similarities between the structure and function of cultured cells *versus* those found in native tissues. The ability of cells grown in 3D cultures to more closely mimic those grown in native environments[24] makes them more effective for use in drug screening preclinical trials.

The use of 3D drug screening techniques for neural tissues is still in its early stage, but substantial research has been done on its use in drug screening for cancer treatment and other tissues[57], using similar principles. For example, Imamura et al.[7] compared the use of 2D *versus* 3D culture models to test the effectiveness of paclitaxel, doxorubicin, and 5-fluorouracil in the treatment of breast cancer[7]. In their study, breast cancer cells on 3D culture plates, which more closely mimicked *in vivo* conditions, tended to form more dense multicellular spheroids when compared to the 2D cultures. The formation of these denser spheroids made the breast cancer cell lines grown in 3D cultures more resistant to both paclitaxel and doxorubicin when compared to 2D cultures in drug sensitivity studies. A second study by DesRochers et al.[8] sought to model nephrotoxicity in 3D cultures while comparing them to 2D cultures. Human renal cortical epithelial cells were cultured in a 3D matrix consisting of a 1:1 ratio of Matrigel to rat tail collagen. It was shown that cells cultured in the 3D matrix more closely mimicked an *in vivo*-like phenotype when compared to 2D cultures of the same cell type. Both the 3D and 2D cultures were exposed to three compounds known to cause nephrotoxicity *in vivo*, cisplatin, gentamicin, and doxorubicin, to determine the concentration of each compound that induces 50% toxicity in the cultured cells LD_{50}. LD_{50} levels showed significantly increased sensitivity to the nephrotoxins in the 3D culture when compared to the 2D culture, indicating that the 3D model is more useful for the detection of nephrotoxicity and drug screening, as it more closely mimics *in vivo* tissues. 3D models for other tissues have shown significant differences in phenotype, when compared to 2D cultures[58–60], further illustrating the benefit of using 3D engineered microenvironments for the screening of anti-inflammatory compounds in the treatment of neurodegenerative diseases.

One obstacle that must be overcome to fully utilize 3D tissue models is to create a bioactive scaffold with specific biological molecules that will better mimic native environments and enhance cell growth. Synthetic scaffolds, while easy to produce, are biologically inert and do not interact with cultured cells in chemically or biologically beneficial ways. It is possible to chemically modify 3D scaffolds and drug screening platforms to include growth factors, proteins, signaling molecules, and other chemical entities to better mimic native tissues. Examples of molecules used in tissue engineering applications are vascular endothelial growth factor (VEGF)[61] and platelet derived growth factor (PDGF)[62], which stimulate blood vessel formation and encourage normal cell growth and division, and the peptide sequence arginine-glycine-aspartic acid[63] (RGD), which is used to improve cell attachment and encourage regular cell behavior. Molecules, such as collagen and laminin may also be included in 3D scaffolds to design matrix that more closely mimics the ECM of neural cells[64].

3. Chemical modification of scaffold materials

An advantage of using 3D tissue engineering scaffolds for drug screening applications is the ability to customize a cell's micro-environment by applying chemical modifications to the scaffold. These modifications allow the creation of a specific cellular niche that enables the user to create a microenvironment with similarities to natural tissues. By customizing the environment in which cells are cultured, it is possible to encourage cellular phenotype and behavior that is comparable to those found *in vivo*. Advances in organic chemistry principles, such as click chemistry, have made the chemical modification of 3D polymeric scaffolds simple and effective in the creation of customized cellular niches to be used in drug screening applications.

3.1. *Chemical modification using click chemistry*

Since the emergence of click chemistry in 2001, it has become a promising technique to engineer the architecture and function of 3D materials[65]. The term click chemistry was introduced in 2001 and was used to describe chemical reactions that are high-yield, with by-products removable without chromatography, regiospecific and stereospecific; can be conducted in aqueous or benign reaction conditions, are orthogonal to other organic synthesis reactions, and are amendable to a wide variety of starting

compounds[66]. Of the click reactions known to date, the most common for use in scaffold modification are alkyne-azide cycloadditions (AAC), Diels-Alder reactions (DA), thiol-ene coupling, and thiol-Michael additions[63,67,68]. The thiol-mediated reactions are particularly useful as their application for surface modification and biofunctionalization of polymers has become commonplace[63].

A study performed by Luo et al.[69] sought to demonstrate the effectiveness of a thiol-maleimide click reaction for immobilizing a glycine-arginine-glycine-aspartic acid-serine peptide sequence (a fibronectin peptide fragment) onto a 3D agarose hydrogel. A photolabile matrix was created by covalent modification of agarose with *S*-2-nitrobenzyl-cysteine, which readily release free thiols when exposed to UV light. These free thiols are then able to react with thiol-reactive biomolecules through Michael addition. Free thiols were reacted with a maleimide-terminated RGD peptide sequence to immobilize it on to the agarose hydrogel. This agarose hydrogel functionalized with an immobilized RGD sequence was shown to promote the extension and migration of primary rat dorsal root ganglia cell *in vitro*[63,69]. Another application of the thiol-maleimide click reaction was performed by Aizawa et al.[61] for the functionalization of an agarose-sulphide hydrogel with VEGF165 gradients. Primary endothelial cells cultured within these hydrogels exhibited tip and stalk cell morphologies as seen *in vivo*[61]. Using a similar reaction, Aizawa et al.[62] immobilized PDGF-AA on thiol-containing channels in an agarose hydrogel. This model was able to preferentially differentiate neural stem/progenitor cells into oligodendrocytes, when they were cultured on the agarose/PDFG-AA hybrid[62,70]. The success of these three models indicate the potential to more closely mimic *in vivo* conditions by functionalizing 3D scaffolds using click chemistry.

A thiol-ene mediated click reaction has also been successfully employed to pattern an RGD peptide sequence onto a PEG hydrogel. These immobilized RGD sequences also proved to influence cell morphology and behavior of cultured cells to behave more like their *in vivo* counterparts[71,72]. It has also been shown that exploitation of thiol-mediated reactions to immobilize multiple peptide sequences onto a single hydrogel allows the creation of more customizable cellular microenvironments for drug screening purposes[71–73]. A benefit of utilizing the thiol-ene is that essentially any functional group chemically linked to a small molecule thiol can be used in a thiol-ene reaction to produce a functionalized scaffold[66]. This has tremendous value as it may become a means to better mimic *in vivo* conditions using 3D scaffolds and tissue engineering principles.

The ability to simulate *in vivo* environments with appropriate cell phenotype and behavior by utilizing principles of click chemistry and compound immobilization may provide superior models for drug screening trials.

4. Drug delivery methods

Another significant challenge to overcome in the treatment of neurodegenerative diseases in the CNS is the delivery of therapeutics across the blood–brain barrier (BBB). The BBB is formed from a series of tight junctions mainly composed of endothelial cells, while other cell types such as astrocytes, pericytes, macrophage, fibroblasts, neuronal cells, basal membranes, and microglia are also included[74]. The purpose of the BBB is to protect the brain from pathogens and disease by restricting the passage of most substances into the CNS[75,76]. In fact, it is estimated that nearly 100% of all large molecule drugs and nearly 98% of all small molecule drugs do not freely cross the BBB without assistance[77–80]. With no direct way to transport the majority of pharmaceuticals across the BBB, it has become increasingly important to apply tissue engineering principles to create novel drug delivery vehicles for the treatment of neurodegenerative diseases.

3D scaffolds can also be effectively used in the treatment of neurodegenerative diseases as vehicles for the delivery of anti-inflammatory therapeutics to sites of chronic inflammation following implantation to the brain. There are multiple ways in which these scaffolds may be utilized, but two of the more common methods of drug delivery from 3D scaffolds are diffusion-based methods and immobilized drug delivery systems. These two systems are different in how drugs are loaded to the scaffold. With diffusion-based methods, the release of desired drugs is regulated by the properties of the scaffold while immobilized systems utilize covalent bonding of drugs directly to the interior/exterior of the scaffold surface. Immobilized drug delivery rate is then determined by the degradation rate of the 3D material[9].

4.1. *Diffusion-based methods*

One prevalent method of diffusion-based drug delivery is the direct loading of the scaffold for delivery. This method involves loading of bioactive compounds directly into the scaffold during gelation. Drug release rates are then determined by concentration gradients and the intrinsic properties of the fabricated scaffold, which affect the diffusion rate of the drug out of the scaffold. An experiment by Burdick et al.[81] used a PEG hydrogel-based delivery system for the delivery of ciliary neurotrophic factor (CNTF) to stimulate neurite outgrowth after disruption of central nervous system tissues. Human CNTF was incorporated into the polymer before curing of the hydrogel, and 3D hydrogels with CNTF inside were formed after polymerization. It was shown experimentally that the PEG hydrogels had an initial 'burst' of CNTF, followed by a more sustained release of the growth factor ranging from 21 to 74 days, depending on mechanical properties of the hydrogel. Tests were then run to determine whether or not the CNTF released from the scaffold retained its biological activity, by exposing retinal explants to the released neurotrophins to enhance neurite outgrowth. The CNTF retained its biological activity as evidenced by inducing neurite outgrowth in retinal explants when compared to control retinal explants without exposure to CNTF[81]. Other studies have also been done to determine the release profiles and effects of other bioactive compounds on CNS models when released from 3D scaffolds[82–84]. These 3D scaffolds call for direct implantation for local delivery of therapeutics to the brain, but due to their small size and physical properties, they require only small disruptions of the BBB to be effective. In some cases, it is possible to inject uncured scaffolds directly into the brain, to allow the polymer to solidify in its target area. A benefit of this approach is minimal disruption of the BBB[10,85].

A second diffusion-based method is through the encapsulation of bioactive compounds into microspheres/nanocarriers first, which can then be integrated into tissue engineered scaffolds or function independently as drug delivery devices. Synthetic polymers are commonly used to encapsulate bioactive compounds, and can be formed into microspheres/nanocarriers, using established methods[86]. A study by Wang et al.[11] determined the release profile of BDNF and VEGF released from a poly(lactic-*co*-glycolic acid)

(PLGA) microsphere system for drug delivery applications. Unlike the direct use of scaffold based delivery systems, the PLGA microsphere system exhibited no initial 'burst' of compound, but provided a slow and mostly linear release profile during the 6-day testing period. The amount of encapsulated BDNF and VEGF released was estimated to be 20%–30% of the total amount contained in the microspheres. The neuron cell survival rate determined by the activity of the released BDNF and VEGF was then verified by treating neurons exposed to glutamate with the released growth factors; with growth factor controls. It has been shown that growth factors incorporated in microspheres did not lose their biological activity upon release, when compared to growth factor controls. Similar microsphere/nanocarrier devices have been implemented for the release of anti-inflammatory agents in neural tissues for the treatment of neurodegenerative disease[87,88], delivery of doxorubicin for the treatment of liver cancer[89], and the delivery of paclitaxel to the lymphatic system for cancer treatment[90], including brain tumors[91]. These microsphere/nanocarrier drug delivery vehicles can be administered locally with small disruptions to the BBB[85], or systemically without BBB disruption by modifying the polymer surface to make them more BBB permeable[92–94].

Delivery systems have also been designed to combine the advantages of scaffold-based delivery with microsphere-based delivery to further customize compound release profiles for specific drug delivery applications. The combination of scaffolds with various microspheres makes it possible to design a delivery that can release multiple bioactive compounds with differing release profiles. This model is an ideal method when treatment with multiple bioactive compounds is beneficial. An example of this method was used by Richardson et al.[12] for the initiation of angiogenesis in a rat model. A polymeric system consisting of a porous poly(lactide-*co*-glycolide) (PLG) scaffold containing VEGF, with PLG microspheres containing PDGF incorporated inside the scaffold, was designed for the dual delivery of both growth factors simultaneously. The results from this experiment showed distinct release profiles for each growth factor from the combined system as well as an increase in blood vessel density and maturation, when compared to single growth factor treatment models. This type of multiplex model has also shown promise in the treatment of neurological disorders[81].

4.2. *Immobilized drug delivery*

A third approach in the delivery of therapeutics *via* 3D scaffolds is the immobilization of drugs chemically onto the scaffold surface. This process often involves covalent bonding between the desired drug and the scaffold itself. This immobilization technique can provide more control over drug delivery, as the release rate is primarily modulated by enzymatic or chemical cleavage of the polymer-drug bond[95]. One model of particular interest in the treatment of neuroinflammation and neurodegenerative diseases was put forth by Nuttelman et al.[96] when they covalently attached dexamethasone, a known anti-inflammatory agent, to a poly(ethylene-glycol) (PEG) scaffold. The covalent attachment was facilitated *via* a lactide linkage, with the number of linkages directly influencing the release rate of the drug from the PEG scaffold. As the number of lactide linkages between the drug and scaffold increased the drug release rate also increased. Dexamethasone was likewise shown to preserve its biological activity[96]. Covalent attachment was used by Chun et al.[10] as well to tether paclitaxel, an anti-tumor drug investigated for use in the treatment of brain tumors, to a poly(organophosphazene) polymer. This paclitaxel-polymer conjugate proved to be more effective in treating tumors long term, when compared to free paclitaxel *in vivo*. The authors attributed this increased *in vivo* activity to the controlled and sustained release of paclitaxel through the hydrolytic cleavage of the paclitaxel-polymer bond[10]. Similar immobilization approaches have been used to adhere growth factors on to polymeric scaffolds for prolonged delivery to neural cells[97–99].

5. Future directions

To improve the use of 3D scaffolds for drug screening and drug delivery purposes, it is required to alter the chemical and biophysical properties of the materials to better suite of the needs of the cultured cells and native tissues. More recently, tissue engineering has evolved to include decellularization techniques to create 3D materials from cell extracellular matrix (ECM) materials complete with growth factors, proteins, and signaling molecules[26,100]. Though current pharmaceutical research with decellularized tissues has often involved matrix modification with natural or synthetic materials[101,102] to re-condition the ECM, there is a potential to retain native scaffold structures for the generation of tissue engineering products, which can be used in compound screening and drug delivery applications.

Decellularization, the removal of cells from tissues or organs, is a method which has been successfully used in aspects of tissue engineering and regenerative medicine for application in transplantation, drug development, and personlized medicine[26,27]. Common methods of decellularization involve a combination of physical and chemical treatments including agitation, freeze-thaw cycles, trypsin, and detergents. Ideally, decellularization will effectively remove all cellular and nuclear materials while minimizing damage to the ECM scaffold's tissue-specific microarchitecture or three-dimensional texture[26,27].

The tissue-specific ECM scaffold's microarchitecture is composed of extracellular components (collagens, glycosaminoglycans, elastin, fibrin, etc.) and growth factors in their proper spatial distribution and ratios. The tissue-specific microenvironment allows for more efficient cell proliferation, attachment, and differentiation[27]. Retention of these tissue-specific microenvironments seems to allow natural ECM scaffolds to be superior to their synthetic counterparts[27,32,103–105]. Further, a key benefit of decellularization is retention of innate vascular networks essential for oxygen and nutrient delivery[100]. An overarching challenge to 3D cell culture systems is the delivery of nutrients and disposal of waste. Cellular spheroids, for example, are a simple but useful 3D tissue model that is limited to a few hundred micrometers, beyond which, a necrotic core develops[106]. Naturally derived ECM scaffolds with retained vasculature hold promise for increasing the size at which tissue engineering can occur. Additionally, as components of the ECM are normally conserved across species, xenogeneic ECM scaffolds can be developed from a variety sources and well tolerated by seeded cells[26]. In review, naturally-derived ECM scaffolds developed *via* decellularization have the potential to show appropriate microarchitecture and functional mechanical properties; be compatible, bioactive, cell supportive, readily available, and inexpensive, as well as present functional vasculature for oxygen and nutrient delivery[27].

The development and use of decellularization in tissue engineering for drug development and screening purposes is ongoing.

Perhaps the most widely used naturally-derived ECM scaffold, Matrigel (reconstituted basement membrane) is processed from the Engelbreth-Holm-Swarm (EHS) sarcoma to produce a soluble and sterile extract that forms a 3D gel[107]. Because Matrigel is derived from a tumor of the basement membrane, and promotes cell growth, it is particularly beneficial in drug screening and development[108–110]. The production of 3D cell cultures using ECM scaffolds developed through decellularization has been, or is currently being, demonstrated in cardiac, adipose, hepatic, pleural, vascular, skeletal muscle, neuronal, and renal tissues[111–118].

An extremely exciting approach to drug testing using tissue engineering is the creation of organoids and organ-on-a-chip/body-on-a-chip systems[111,112,117,119–121]. Organoids, simplified miniature organs, can be produced from primary tissues as well as embryonic stem cells or induced pluripotent stem cells[20]. Because the behavior of cells are controlled by the microenvironment, organoids frequently utilize naturally-derived or synthetic ECM substrates, small molecules, and growth factors to fine tune the self-renewal/differentiation of stem cells and self-assembly of cells in organoids[122]. As a near 3D physiological model, organoids are capable of biological processes including tissue renewal, mutation, and metabolism, and conversion of prodrugs into an active metabolite[20,123]. Body-on-a-chip systems have been able to capitalize on the interplay of several connected organoids to closely replicate size relationships of organs, blood distribution, and blood flow represented in human physiology[111,112,123]. Additionally, using a patient's own cells to create organoids used in body-on-chip systems will lead to advances in personalized medicine and potentially eliminate the use of animals in preclinical trials[111,123].

6. Conclusions

In summary, the use of the tissue engineering model and 3D mimicking materials and their applications will enhance the fields of pharmaceutical science and pharmacology as pharmaceutical scientists strive to solve the neurodegenerative disease problem. Used as drug screening and drug delivery tools, 3D models may open the door to improved and novel treatments that will promote the recovery and quality of life of individuals afflicted with these diseases. More recent advancements in the tissue engineering model using decellularization techniques will further augment drug screening models by providing better tissue mimics to experiment with chemical effects on neural cells and toxicity in connected tissues. There is much work that needs to be done in order to cure these diseases, but application of the tissue engineering model will go a long way in bridging the gap between treating these diseases and striving to manage the symptoms expressed in individuals.

Acknowledgments

This work was supported by the faculty startup fund from the Utah State University and the subcontract of a NIH grant (R21 CA190024) from the Houston Methodist Research Institute.

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