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.

© 2018 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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. 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. Promising results in the treatment of many neurodegenerative diseases have been obtained during preliminary trials by mitigating neural inflammation affiliated with these diseases. 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, the results of which have provided unique platforms for the enhancement of drug delivery to areas of need.

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, microglia are 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.

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. 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. 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.

This heightened inflammatory response and state of reactive microgliosis has been commonly observed in neurodegenerative diseases such as Parkinson’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis, and traumatic brain injury. 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](image_url) 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” ("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. 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. 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. The organoid model is also being used for the study of the Zika virus and its effects on human brain development.

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 (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, 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 (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. It has been shown that cells respond to the resistance, or stiffness, of the substrate on which they are cultured, with the substrate stiffness directly affecting the proliferation and differentiation of stem cells to neural cells.

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. Acceptable tissue engineering models of the human brain should include microglial cells to more accurately reflect inflammatory pathways in the brain.

![Figure 2](image-url)
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. 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 E2 (PGE2), which is the major prostaglandin associated with the presence of inflammation in the brain and spinal cord. Several NSAIDs have shown promise for the attenuation of the inflammatory response by reducing the levels of PGE2 in circulation, in animal models, once an insult to the brain has been experienced (Table 1). 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 and Parkinson’s disease, 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 *Ulma 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). 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*. A thorough review by Pengestuti and Kim 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. 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. Additionally, multiple compounds isolated from the root bark of *Lycium chinense* have shown to suppress the production of NO in LPS-induced BV2 cells. 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. 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. Due to the vinyl ether moiety located on the first carbon of the glycerol backbone, it is thought that plasmalogens have antioxidant effects 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 PGE2 level by 50% in rats (1 nmol/L)                       | Approved drug                 |
| Piroxicam (NSAID)   | Reduced the PGE2 level by 50% (0.1 µmol/L)                             | Approved drug                 |
| Flurbiprofen (NSAID) | Reduced the PGE2 level by 50% (0.1 µmol/L)                             | Approved drug                 |
| Paracetamol (NSAID) | Reduced the PGE2 level by 50% (7.6 µmol/L)                             | Approved drug                 |
| Acetylsalicylic acid (NSAID)| Reduced the PGE2 level by 50% (10 µmol/L) | Approved drug                 |
| NS-398 (NSAID)      | COX-2 inhibitor: reduced the PGE2 levels by 50% (1–5 nmol/L)          | Chemical approved for research |
| Torilin             | Reduced neurotoxic factors after LPS exposure in murine BV2 microglial cells | Natural product/drug candidate |
| Macelignan          | Reduced neurotoxic factors after LPS exposure in rat microglial cultures | Natural product/drug candidate |
| Marine algae        | Reduced pro-inflammatory mediators in murine BV2 and HT22 cell lines  | Natural product/drug candidate |
| EHT                 | Reduced pro-inflammatory mediators in cultured primary microglia      | Natural product/drug candidate |
| Lycium chinense     | Suppress production of NO in BV2 cells                                | Natural product/drug candidate |
| Plasmalogens        | Attenuation of microglia activation and of pro-inflammatory mediators in mice PFC | Natural product/drug candidate |
| PHPB                | Reduce levels of pro-inflammatory intermediates in mice               | Phase II clinical trials       |
A novel neuroprotective compound, potassium 2-(1-hydroxy-pentyl)-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. PHPB is currently in phase II clinical trial for ischemic stroke in China.

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. 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; 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. 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. 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 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, using similar principles. For example, Imamura et al. compared the use of 2D versus 3D culture models to test the effectiveness of paclitaxel, doxorubicin, and 5-fluourouracil in the treatment of breast cancer. 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. 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<sub>50</sub> 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, 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) and platelet derived growth factor (PDGF), which stimulate blood vessel formation and encourage normal cell growth and division, and the peptide sequence arginine-glycine-aspartic acid (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.

### 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 microenvironment 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. 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. 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. The thiol-mediated reactions are particularly useful as their application for surface modification and biofunctionalization of polymers has become commonplace.

A study performed by Luo et al. 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. This model was able to preferentially differentiate neural stem/progenitor cells into oligodendrocytes, when they were cultured on 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. Another application of the thiol-maleimide click reaction was performed by Aizawa et al. 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. Using a similar reaction, Aizawa et al. immobilized PDGF-AA on thiol-containing channels in an agarose hydrogel. This model was able to influence cell morphology and behavior of cultured cells to behave more like their in vivo counterparts. 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. 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. 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. The purpose of the BBB is to protect the brain from pathogens and disease by restricting the passage of most substances into the CNS. 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. 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 is then determined by the degradation rate of the 3D material.

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. 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. 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. 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.

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. A study by Wang et al. 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 microparticles. 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, delivery of doxorubicin for the treatment of liver cancer, and the delivery of paclitaxel to the lymphatic system for cancer treatment, including brain tumors. These microsphere/nanocarrier drug delivery vehicles can be administered locally with small disruptions to the BBB, or systemically without BBB disruption by modifying the polymer surface to make them more BBB permeable.

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. 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.

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. One model of particular interest in the treatment of neuroinflammation and neurodegenerative diseases was put forth by Nuttelman et al. 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 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. Similar immobilization approaches have been used to adhere growth factors to polymeric scaffolds for prolonged delivery to neural cells.

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 suit 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. Though current pharmaceutical research with decellularized tissues has often involved matrix modification with natural or synthetic materials 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 personalized medicine. 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.

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. Retention of these tissue-specific microenvironments seems to allow natural ECM scaffolds to be superior to their synthetic counterparts. Further, a key benefit of decellularization is retention of innate vascular networks essential for oxygen and nutrient delivery. 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. 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. 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.

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 gel111.112.117,119. Because Matrigel is derived from a tumor of the basement membrane, and promotes cell growth, it is particularly beneficial in drug screening and development108–110. 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 tissues111–113.

An extremely exciting approach to drug testing using tissue engineering is the creation of organoids and organ-on-a-chip/body-on-a-chip systems111,112,117,119–121. Organoids, simplified miniature organs, can be produced from primary tissues as well as embryonic stem cells or induced pluripotent stem cells20. 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 organoids122. 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 metabolite20,123. 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 representative in human physiology111,112,123. 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 trials111,123.

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.

References

1. Alzheimer's Association. Alzheimer's disease facts and figures. [cited 2017 Oct 7]; 2017. Available from: (https://www.alz.org/facts).
2. Statistics on Parkinson's. Parkinson's Foundation. [cited 2018 Mar 13]. Available from: (http://parkinson.org/Understanding-Parkinsons/Causes-and-Statistics/Statistics).
3. Block ML, Hong JS. Chronic microglial activation and progressive dopaminergic neurotoxicity. Biochem Soc Trans 2007;35:1127–32.
4. Ajmone-Cat MA, Bernardo A, Greco A, Minghetti L. Non-steroidal anti-inflammatory drugs and brain inflammation: effects on microglial functions. Pharmaceuticals 2010;3:1949–65.
5. Ituku M, Katafuchi T, Mawatari S, Noda M, Maitke K, Sugiyama M, et al. Anti-inflammatory/anti-amyloidogenic effects of plasminogenses in lipopolysaccharide-induced neuroinflammation in adult mice. J Neuroinflammation 2012;9:197.
6. Pangestuti R, Kim SK. Neuroprotective effects of marine algae. Mar Drugs 2011;9:803–18.
7. Imamura Y, Mukohara T, Shimono Y, Funakoshi Y, Chayahara N, Toyoda M, et al. Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncol Rep 2015;33:1837–43.
8. DesRochers TM, Suter L, Roth A, Kaplan DL. Bioengineered 3D human kidney tissue, a platform for the determination of nephrotoxicity. PLoS One 2013;8:e59219.
9. Willerth SM, Sakiyama-Elbert SE. Approaches to neural tissue engineering using scaffolds for drug delivery. Adv Drug Deliv Rev 2007;59:325–38.
10. Chun C, Lee SM, Kim SY, Yang HK, Song SC. Thermosensitive poly(organophosphazene)-paclitaxel conjugate gels for antitumor applications. Biomaterials 2009;30:2349–60.
11. Wang Y, Wei YT, Zu ZH, Ju RK, Guo MY, Wang XM, et al. Combination of hyaluronic acid hydrogel scaffold and PLGA microspheres for supporting survival of neural stem cells. Biomaterials 2011;32:1029–34.
12. Richardson TP, Peters MC, Emnett AB, Mooney DJ. Polymeric scaffold for dual growth factor delivery. Nat Biotechnol 2001;19:357–65.
13. Gao HM, Hong JS. Why neurodegenerative diseases are progressive: uncontrollable inflammation drives disease progression. Trends Immunol 2008;29:357–65.
14. Liu B, Hong JS. Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. J Pharmacol Exp Ther 2003;304:1–7.
15. Loane DJ, Byrnes KR. Role of microglia in neurotrauma. Neurotherapeutics 2010;7:566–77.
16. Lull ME, Block ML. Microglial activation and chronic neurodegeneration. Neurotherapeutics 2010;7:354–65.
17. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005;308:1314–8.
18. Wu DC, Ré DB, Nagai M, Ischiropoulos H, Przedborski S. The inflammatory NADPH oxidase enzyme modulates motor neuron degeneration in amyotrophic lateral sclerosis mice. Proc Natl Acad Sci U S A 2006;103:12132–7.
19. Lenzlinger PM, Morganti-Kossmann MC, Learner HL, McIntosh TK. The duality of the inflammatory response to traumatic brain injury. Mol Neurobiol 2001;24:169–81.
20. Fatelahullah A, Tan SH, Barker N. Organoids as an in vitro model of human development and disease. Nat Cell Biol 2016;18:246–54.
21. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, et al. Cerebral organoids model human brain development and microcephaly. Nature 2013;504:373–9.
22. Garcez PP, Loiola EC, Da Costa RM, Higa LM, Trindade P, Delvecchio R, et al. Zika virus impairs growth in human neurosphere and brain organoids. Science 2016;352:816–8.
23. Cugola F, Fernandes I, Russo FB, Freitas BC, Dias JL, Guimarães KP, et al. The Brazilian Zika virus strain causes birth defects in experimental models. Nature 2016;534:267–71.
24. Rimann M, Graf-Hausner U. Synthetic 3D multicellular systems for drug development. Curr Opin Biotechnol 2012;5:803–9.
25. Roohani-Esfahani SI, Newman P, Zreiqat H. Design and fabrication of 3D printed scaffolds with a mechanical strength comparable to cortical bone to repair large bone defects. Sci Rep 2016;6:19468.
26. Gilbert TW, Sellaro TL, Badylah SF. Decellularization of tissues and organs. *Biomaterials* 2006;27:3675–83.

27. Orlando G, Wood KJ, Stratta RJ, Yoo JJ, Atala A, Soker S. Regenerative medicine and organ transplantation: past, present, and future. *Transplantation* 2011;91:1310–7.

28. Georges PC, Miller WJ, Meaney DF, Sawyer ES, Janmey PA. Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures. *Biophys J* 2006;90:3012–8.

29. Leipzig ND, Shoichet MS. The effect of substrate stiffness on adult neural stem cell behavior. *Biomaterials* 2009;30:6867–78.

30. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science* 2005;310:1139–43.

31. Mandal BB, Kundu SC. Cell proliferation and migration in silk fibroin 3D scaffolds. *Biomaterials* 2009;30:2956–65.

32. De Waele J, Reekmans K, Daans J, Goossens H, Berneman Z, Issaoun M. Time- and concentration-dependent neurite outgrowth of human neural progenitor cells on silk fibroin-based scaffolds. *Acta Biomater* 2015;11:122–31.

33. Miller RL, James-Kracke M, Sun GY, Sun AY. Oxidative and inflammatory pathways in Parkinson's disease. *Neurochem Res* 2009;34:55–65.

34. Weissman G. The actions of NSAIDs. *Hosp Pract* 1991;26:60–76.

35. Day RO, Graham GG. Non-steroidal anti-inflammatory drugs (NSAIDs). *BJM* 2013;346:e3195.

36. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. *Arterioscler Thromb Vasc Biol* 2011;31:986–1000.

37. Teeling JL, Cunningham C, Newman TA, Perry VH. The effect of non-steroidal anti-inflammatory agents on behaviourial changes and cytokine production following systemic inflammation: implications for a role of COX-1. *Brain Behav Immun* 2010;24:409–19.

38. Greco A, Ajmone-Cat MA, Nicolini A, Sciuli MG, Minghetti L. NO induces cyclooxygenase-2 and inducible nitric oxide synthase expression in brain macrophages by inhibiting enzymatic activity of cyclooxygenase but not phospholipase and prostaglandin E synthase. *J Neurosci Res* 2003;71:844–52.

39. Ajmone-Cat MA, Nicolini A, Minghetti L. Differential effects of the nonsteroidal antiinflammatory drug flurbiprofen and its nitric oxide-releasing derivative, nitrofuribuprofen, on prostaglandin E2, interleukin-1β, and nitric oxide synthesis by activated microglia. *J Neurosci Res* 2001;66:715–22.

40. Choi Y, Lee MK, Lim SY, Sung SH, Kim YC. Inhibition of inducible NO synthesis, cyclooxygenase-2 and interleukin-1β by tolirin is mediated by mitogen-activated protein kinase in microglial BV2 cells. *Br J Pharmacol* 2009;156:933–40.

41. Jin DQ, Lim CS, Hwang JK, Ha I, Jin HS. Anti-oxidant and anti-inflammatory activities of mucelliginate in murine hippocampal cell line and primary culture of rat microglial cells. *Biochem Biophys Res Commun* 2005;331:1264–9.

42. Jung WK, Ahn YW, Lee SH, Choi YH, Kim SK, Yea SS, et al. Ecklonia cava ethanolic extracts inhibit lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in BV2 microglia via the MAP kinase and NF-κB pathways. *Food Chem Toxicol* 2009;47:410–7.

43. Lim CS, Jin DQ, Sung JY, Lee JH, Choi HG, Ha I, et al. Antioxidant and anti-inflammatory activities of the methanolic extract of *Nehrodomela aculeata* in hippocampal and microglial cells. *Biol Pharm Bull* 2006;29:1212–6.

44. Jin DQ, Lim CS, Sung JY, Choi HG, Ha I, Han JS. Ulva conglobata, a marine algae, has neuroprotective and anti-inflammatory effects in murine hippocampal and microglial cells. *Neurosci Lett* 2006;402:154–8.

45. Lee KW, Im JY, Woo JM, Grosso H, Kim YS, Cristovao AC, et al. Neuroprotective and anti-inflammatory properties of a coffee component in the MPTP model of Parkinson's disease. *Neurotherapeutics* 2013;10:143–53.

46. Yang Y, An Y, Wang W, Du N, Zhang J, Feng Z, et al. Nine compounds from the Root Bark of *Lycium chinense* and their anti-inflammatory activities. *Acta Pharm Sin B* 2017;7:491–5.

47. Zhao C, Hou W, Lei H, Huang L, Wang S, Cui D, et al. Potassium 2-(1-hydroxypropyl)-benzoate attenuates neuroinflammatory responses and upregulates heme oxygenase-1 in systemic lipopolysaccharide-induced inflammation in mice. *Acta Pharm Sin B* 2017;7:470–8.

48. Wenk GL. Neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry* 2003;64(Suppl 9):7–10.

49. Sánchez-Pernaute R, Ferrere A, Cooper O, Yu M, Brownell AL, Isacson O. Selective COX-2 inhibition prevents progressive dopaminergic degeneration in a rat model of Parkinson's disease. *J Neuroinflammation* 2004;1:6.

50. Murphy RC. Free-radical-induced oxidation of arachidonyl plasmalogens phospholipids: antioxidant mechanism and precursor pathway for bioactive eicosanoids. *Chem Res Toxicol* 2001;14:463–72.

51. Luoma AM, Kuo F, Kakici O, Crowther MN, Denninger AR, Avila RL, et al. Plasmalogens phospholipids protect internodal myelin from oxidative damage. *Free Radic Biol Med* 2015;84:296–310.

52. DiMaio JA, Hansen RW, Grabowski HG. The price of innovation: new estimates of drug development costs. *J Health Econ* 2003;22:151–85.

53. Food and Drug Administration. Innovation of stagnation: challenge and opportunity on the critical path to new medical products. [cited 2017 Sep 23]. Available from: (https://www.fda.gov/downloads/scienceresearch/specialtopics/criticalpathinitiative/criticalpathopportunities/ucm1134411.pdf).

54. Breslin S, O'Driscoll L. Three-dimensional cell culture: the missing link in drug discovery. *Drug Discov Today* 2013;18:240–9.

55. Kimlin L, Kassis J, Virador V. 3D printed scaffolds: the precursor in tissue engineering. *Expert Opin Drug Discov* 2012;244:870–2.

56. Nam KH, Smith AS, Lone S, Kwon S, Kim DH. Biomimetic 3D tissue models for advanced high-throughput drug screening. *J Lab Automat 2015;20:201–15.

57. Vandenburgh H, Shansky J, Benesch-Lee F, Barbata V, Reid J, Thorrez L, et al. Drug-screening platform based on the contractility of tissue-engineered muscle. *Muscle Nerve* 2008;37:438–47.

58. Kim K, Ohashi K, Utoh R, Kano K, Okano T. Preserved liver-specific functions of hepatocytes in 3D co-culture with endothelial cell sheets. *Biomaterials* 2012;33:1406–13.

59. Godugu C, Patel AR, Desai U, Andey T, Sams A, Singh M. Algimatrix® based 3D cell culture system as an in-vitro tumor model for anticancer studies. *PLoS One* 2013;8.e53708.

60. Aizawa Y, Wylie R, Shoichet M. Endothelial cell guidance in 3D patterned scaffolds. *Adv Mater* 2010;22:4831–5.

61. Aizawa Y, Leipertz N, Zahir T, Shoichet M. The effect of immobilized platelet derived growth factor AA on neural stem/progenitor cell differentiation on cell-adhesive hydrogels. *Biomaterials* 2008;29:4676–83.

62. Nimmo CM, Shoichet MS. Regenerative biomaterials that “click”: simple, aqueous-based protocols for hydrogel synthesis, surface immobilization, and 3D patterning. *Bioconj Chem* 2011;22:2199–209.

63. Suri S, Schmidt CE. Cell-laden hydrogel constructs of hyaluronic acid, collagen, and laminin for neural tissue engineering. *Tissue Eng Part A* 2010;16:1703–16.

64. Such GK, Johnston AP, Liang K, Caruso F. Synthesis and functionalization of nanogengineered materials using click chemistry. *Prog Poly Sci* 2012;37:985–1003.

65. Hoyle CE, Bowman CN. Thiol-ene click chemistry. *Angew Chem Int Ed Engl* 2010;49:1540–73.

66. Xi W, Scott TF, Kloxin CJ, Bowman CN. Click chemistry in materials science. *Adv Funct Mater* 2014;24:2572–90.

67. Azagarsamy MA, Anseth KS. Biothrogonal click chemistry: an indispensable tool to create multifaceted cell culture scaffolds. *ACS Macro Lett* 2013;2:5–9.

68. Luo Y, Shoichet MS. A photolabile hydrogel for guided three-dimensional cell growth and migration. *Nat Mater* 2004;3:249–53.
3D tissue engineering, an emerging technique for pharmaceutical research

70. Jiang Y, Chen J, Deng C, Saaronen EJ, Zhong Z. Click hydrogels, microgels and nanogels: emerging platforms for drug delivery and tissue engineering. *Biomaterials* 2014;35:4969–85.

71. DeForest CA, Polizotti BD, Anseth KS. Sequential click reactions for synthesizing micro-patterned three-dimensional cell microenvironments. *Nat Mater* 2009;8:659–64.

72. DeForest CA, Sims EA, Anseth KS. Peptide-functionalized click hydrogels with independently tunable mechanics and chemical functionality for 3D cell culture. *Chem Mater* 2010;24:4783–90.

73. Wylie RG, Ahsan S, Aizawa Y, Maxwell KL, Morshead CM, Engelhardt B. Development of the blood-brain barrier: bottleneck in brain drug delivery. *Integr Biol* 2009;28:271–84.

74. Zhao X, Chen R, Liu M, Feng J, Chen J, Hu K. Remodeling the blood-brain barrier microenvironment by natural products for brain tumor therapy. *Acta Pharmac Sin B* 2017;7:541–53.

75. Engelhardt B. Development of the blood-brain barrier. *Cell Tissue Res* 2003;314:119–29.

76. Reece JB, Unry LA, Cain ML, Wasserman SA, Minovsky PV, Jackson RB. In: *Campbell Biology*, 9th ed. New York: Pearson; 2011. p. 1066.

77. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx* 2005;2:3–14.

78. Pardridge WM. Blood–brain barrier delivery. *Drug Discov Today* 2007;12:54–61.

79. Pardridge WM. Drug transport across the blood-brain barrier. *J Cereb Blood Flow Metab* 2012;32:1959–72.

80. Patel MM, Goyal BR, Bhadada SV, Bhatt JS, Amin AF. Getting into the brain: approaches to enhance brain drug delivery. *CNS Drugs* 2009;23:35–38.

81. Burdick JA, Ward M, Liang E, Young MJ, Langer R. Stimulation of neurite outgrowth by neurotrophins delivered from degradable hydrogels. *Biomaterials* 2006;27:452–9.

82. Barras FM, Pasche P, Bouche N, Aebischer P, Zurn AD. Glial cell line-derived neurotrophic factor released by synthetic guidance channels promotes facial nerve regeneration in the rat. *J Neurosci Res* 2002;70:746–55.

83. Bloch J, Fine EG, Bouche N, Zurn AD, Aebischer P. Nerve growth factor- and neurotrophin-3-releasing guidance channels promote regeneration of the transected rat dorsal root. *Exp Neurol* 2001;172:425–32.

84. Fine EG, Decoster I, Papaloizou M, Zurn AD, Aebischer P. GDNF and NGF released by synthetic guidance channels support sciatic nerve regeneration across a long gap. *Eur J Neurosci* 2002;15:589–601.

85. Donaghe IE, Tam R, Selton MV, Shoichet MS. Cell and biomolecule delivery for tissue repair and regeneration in the central nervous system. *J Control Release* 2014;190:219–27.

86. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers* 2013;5:1377–97.

87. Kim DH, Martin DC. Sustained release of dexamethasone from hydrophilic matrices using PLGA nanoparticles for neural drug delivery. *Biomaterials* 2006;27:3031–7.

88. Menei P, Montero-Menei C, Venier MC, Benoit JP. Drug delivery into the brain using poly(lactide-co-glycolide) microspheres. *Expert Opin Drug Deliv* 2005;2:363–76.

89. Hong K, Khwaja A, Liapi E, Torbenson MS, Georgiades CS, Geschwind JF. New intra-arterial drug delivery system for the treatment of liver cancer: preclinical assessment in a rabbit model of liver cancer. *Clin Cancer Res* 2006;12:2563–7.

90. Liu J, Meinsner D, Kwong E, Wu XY, Johnston MR. A novel trans-lymphatic drug delivery system: implantable gelatin sponge impregnated with PLGA-paclitaxel microspheres. *Biomaterials* 2007;28:3236–44.

91. Ranganath SH, Kee I, Krantz WB, Chow PK, Wang CH. Hydrogel matrix entrapping PLGA-paclitaxel microspheres: drug delivery with near zero-order release and implantability advantages for malignant brain tumour chemotherapy. *Pharm Res* 2009;26:2101–14.

92. Rao KS, Reddy MK, Horning JL, Labhasetwar V. TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs. *Biomaterials* 2008;29:4429–38.

93. Liu M, Li H, Luo G, Liu Q, Wang Y. Pharmacokinetics and biodistribution of surface modification polymeric nanoparticles. *Arch Pharm Res* 2008;31:547–54.

94. Patel T, Zhou J, Piepmeier JM, Saltzman WM. Polymeric nanoparticles for drug delivery to the central nervous system. *Adv Drug Deliv Rev* 2012;64:701–5.

95. Bhattachar N, Gunn J, Zhang M. Chitosan-based hydrogels for controlled, localized drug delivery. *Adv Drug Deliv Rev* 2010;62:83–99.

96. Nuttelma CR, Tripodi MC, Anseth KS. Dexamethasone-functionalized gels induce osteogenic differentiation of encapsulated hMSCs. *J Biomed Mater Res A* 2006;76A:183–95.

97. Gomez N, Yu L, Chen S, Schmidt CE. Immobilized nerve growth factor and microtopography have different effects on polarization versus axon elongation in hippocampal cells in culture. *Biomaterials* 2007;28:271–84.

98. Kapur TA, Shoichet MS. Chemically-bound nerve growth factor for neural tissue engineering applications. *J Biomater Sci Polym Ed* 2003;14:383–94.

99. Sakiyama-Elbert SE, Panitch A, Hubbell JA. Development of growth factor fusion proteins for cell-triggered drug delivery. *FASEB J* 2001;15:1300–2.

100. Badyal SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. *Annu Rev Biomed Eng* 2011;13:27–53.

101. Skardal A, Smith L, Bharadwaj S, Atala A, Soker S, Zhang Y. Tissue specific synthetic ECM hydrogels for 3-D in vitro maintenance of hepatocyte function. *Biomaterials* 2012;33:4565–75.

102. Skardal A, Murphy SV, Devarasetty M, Mead I, Kang HW, Seil YJ, et al. Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. *Sci Rep* 2017;7:8837.

103. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials* 2012;32:3233–43.

104. Uriel S, Labay E, Francis-Sedlak M, Moya ML, Weischelbaum RR, Ervin N, et al. Extraction and assembly of tissue-derived gels for cell culture and tissue engineering. *Tissue Eng Part C Methods* 2008;15:309–21.

105. Ribatti D, Conconi MT, Nico B, Baiguera S, Corsi P, Parnigotto PP, et al. Angiogenic response induced by acellular brain scaffolds grafted onto the chick embryo chorioallantoic membrane. *Brain Res* 2009;1289:9–15.

106. Powers MJ, Janigian DM, Wack KE, Baker CS, Stolz DB, Griffith LG. Functional behavior of primary rat liver cells in a three-dimensional perfused microarray bioreactor. *Tissue Eng* 2004;10:499–513.

107. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. *Semin Cancer Biol* 2005;15:378–86.

108. Sung JH, Shuler ML. A micro cell culture analog (µCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. *Lab Chip* 2009;9:1385–94.

109. Hansen A, Eder A, Bönstrup M, Flato M, Mewe M, Schaaf S, et al. Development of a drug screening platform based on engineered heart tissue. *Circ Res* 2010;107:35–44.

110. Serebriiskii I, Castelli-Cros R, Lamb A, Golenis EA, Cukierman E. Fibroblast-derived 3D matrix differentially regulates the growth and drug-responsiveness of human cancer cells. *Matrix Biol* 2008;27:573–85.

111. Skardal A, Shupe T, Atala A. Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling. *Drug Discov Today* 2016;21:1399–411.

112. Abaci HE, Shuler ML. Human-on-a-chip design strategies and principles for physiologically based pharmacokinetics/pharmacodynamics modeling. *Integr Biol* 2015;7:383–91.
113. Iyer RK, Chiu LL, Reis LA, Radisic M. Engineered cardiac tissues. *Curr Opin Biotechnol* 2011;22:706–14.

114. Dunne LW, Huang Z, Meng W, Fan X, Zhang N, Zhang Q, et al. Human decellularized adipose tissue scaffold as a model for breast cancer cell growth and drug treatments. *Biomaterials* 2014;35:4940–9.

115. Hussein KH, Park KM, Teotia PK, Yang JW, Kim HM, Hong SH, et al. Fabrication of a biodegradable xenoantigen-free rat liver scaffold for potential drug screening applications. *Transplant Proc* 2013;45:3092–6.

116. Sart S, Yan Y, Li Y, Lochner E, Zeng C, Ma T, et al. Crosslinking of extracellular matrix scaffolds derived from pluripotent stem cell aggregates modulates neural differentiation. *Acta Biomater* 2016;30:222–32.

117. Shuler ML, Hickman JJ. Toward *in vitro* models of brain structure and function. *Proc Natl Acad Sci U S A* 2014;111:13682–3.

118. Wassenaar JW, Boss GR, Christman KL. Decellularized skeletal muscle as an *in vitro* model for studying drug–extracellular matrix interactions. *Biomaterials* 2015;64:108–14.

119. Baptista PM, Siddiqui MM, Lozier G, Rodriguez SR, Atala A, Soker S. The use of whole organ decellularization for the generation of a vascularized liver organoid. *Hepatology* 2011;53:604–17.

120. Fong EL, Toh TB, Yu H, Chow EK. 3D culture as a clinically relevant model for personalized medicine. *SLAS Technol* 2017;22:245–53.

121. Fang Y, Eglen RM. Three-dimensional cell cultures in drug discovery and development. *SLAS Discov* 2017;22:456–72.

122. Yin X, Mead BE, Safaee H, Langer R, Karp JM, Levy O. Stem cell organoid engineering. *Cell Stem Cell* 2016;18:25–38.

123. Mahler GJ, Esch MB, Stokol T, Hickman JJ, Shuler ML. Body-on-a-chip systems for animal-free toxicity testing. *Altern Lab Anim* 2016;44:469–78.