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Biomaterial applications in neural therapy and repair

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Abstract

The use of biomaterials, such as hydrogels, as a scaffold to deliver cells and drugs is becoming increasingly common to treat neurological conditions, including stroke. With a limited intrinsic ability to regenerate after injury, innovative tissue engineering strategies have shown the potential of biomaterials in facilitating neural tissue regeneration and functional recovery. Using biomaterials can not only promote the survival and integration of transplanted cells in the existing circuitry, but also support controlled site specific delivery of therapeutic drugs. This review aims to provide the reader an understanding of the brain tissue microenvironment after injury, biomaterial criteria that support tissue repair, commonly used natural and synthetic biomaterials, benefits of incorporating cells and neurotrophic factors, as well as the potential of endogenous neurogenesis in repairing the injured brain.

Keywords: Biomaterial, Hydrogel, Microparticles, Stroke, Cell therapy, Drug delivery, Brain, ECM, Neurogenesis, Tissue engineering, Biocompatibility

Background

Stroke is the leading cause of adult disability affecting nearly 800,000 Americans each year, with ischemic stroke accounting for 80 % of all cases [1]. With the increasing incidence of stroke, and declining mortality, the number of disabled stroke survivors is expected to increase [2]. While patients currently rely on physical therapy to restore motor function after stroke, these improvements are modulated through existing brain circuitry [3], and not through replacing lost cells and tissue with up to 30 % of the stroke patients remaining permanently disabled even with intensive task specific training [4, 5]. In other words, there is a loss of functional tissue after stroke, and thus the physical therapy and rehabilitation is limited to restoring the lost cognitive and physiological functions. The lack of effective treatments for stroke and other neurological diseases can also be explained by the limited regenerative potential of the central nervous system [6].

Regenerative strategies, such as stem cell therapy, have shown limited success in improving behavioral outcomes, but there is no replacement of the lost tissue [7, 8], and hence a large tissue cavity remains in the brains of stroke survivors [9]. There are 2 major limitations with stem cell therapy. Firstly, there is a large scale loss of the transplanted cells in the days following intracerebral implantation, with survival ranging from 1 to 32 % [10]. Similarly, systemic administration of cells in ischemic rats accumulates cells primarily in internal organs instead of the brain [11]. Secondly, intracerebral cell injection requires transplantation into the peri-infarct region, which is considered to be an active site for cerebral reorganization after stroke [12], and multiple injections into this site can potentially damage the tissue even further. One key parameter that has been shown to affect cell survival and neurogenesis is the regulation of inflammatory cytokines. These cytokines have a detrimental effect on the cells transplanted in close proximity to the cavity [13]. The stroke cavity is surrounded by resident immune cells, i.e. microglia, as well as perivascular macrophages who respond to the injury by releasing inflammatory cytokines [14]. In order to modulate the immune response and improve the outcome of cell therapy, an ideal biomaterial will protect the cellular graft from the host response and interact with the activated immune cells in a positive manner.

Injectable biomaterials can be used as a scaffold to fill the stroke cavity and promote interactions between transplanted material and host tissue [15–18], deliver drugs or growth factors to the damaged tissue, promote

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the attachment and engraftment of transplanted cells, and help recruit host cells to repopulate the lost tissue [19]. Protective hydrogels derived from natural and synthetic polymers can incorporate cells, growth factors, and other therapeutics to enhance the microenvironment and provide controlled release of bioactive molecules. This review will briefly address the complex microenvironment after stroke, ideal characteristics of a biomaterial, natural vs synthetic materials, biomaterial based cell and drug delivery, and lastly, explore the potential of endogenous neurogenesis and cell replacement in brain tissue repair.

Stroke microenvironment

In the very early stages of stroke, adenosine triphosphate (ATP) consumption continues despite insufficient synthesis due to reduced glucose and oxygen flow in the tissue. This leads to a drop in total ATP available to the cells and ionic homeostasis. Severe ischemia results in an excess release of the main excitotoxic neurotransmitter glutamate, which promotes a major influx of calcium into the cells and activates the phospholipases and proteases to degrade proteins and membranes in neuronal cells [20]. After the onset of stroke, the core of the infarct results in an area of complete cell death leading to irreversibly damaged tissue [21]. Cerebral blood flow (CBF) is highly compromised in this region with <20 % of baseline blood flow levels [22].

With fast degradation of the damaged host tissue extracellular matrix (ECM), the cavity is filled with extracellular fluid [23], and transplantation of cells in this region would result in severe loss of the injected graft [13, 24]. The brain tissue surrounding the core, or the ischemic penumbra (IP), contains partially dying cells as well as activated microglia, peripheral macrophages and astrocytes. Microglia, the resident immune cells of the brain constantly monitor the microenvironment and respond to the insult with typical macrophagic roles, such as secretion of cytokines, phagocytosis and antigen presentation. Microglia are activated within minutes of the stroke onset and accumulate surrounding the lesion cavity in the IP. The proliferation of these immune cells peaks at 48–72 h after the ischemia and may last for several weeks depending on the extent of damage [25, 26]. While traditionally these cells were considered to be deleterious and neurotoxic by releasing pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-alpha and Interleukin (IL)-1 [27–29], it has been shown that activated microglia can maintain and support neuronal survival [30, 31] by releasing anti-inflammatory and neurotrophic factors. Microglia have also been shown to promote neurogenesis by guiding the stem cells to the site of injury [32–34].

In addition to traditional immune cells, astrocytes have also been known to express various inflammatory mediators, such as cytokines and chemokines, that mediate the immune response. The astrocytic response after the injury, or reactive gliosis, is characterized by excessive expression of glial fibrillary acidic protein (GFAP), cellular hypertrophy and process extension, creating a glial scar tissue surrounding the lesion [35–37]. It is well known that astrocytes are more resistant to oxygen and glucose deprivation [35], which enables them to survive for a prolonged period in the IP where the vasculature is partially maintained [38]. Astrocytes are involved in a number of activities during ischemia, including regulating the blood brain barrier (BBB), CBF regulation, glutamate and ion homeostasis [38–42]. Although astrocytes limit axon outgrowth by expressing inhibitory molecules (e.g. proteoglycans) and forming a glial scar [43], they are also known to release extracellular matrix proteins, such as thrombospondins 1 and 2, which have been shown to increase synpatogenesis and axonal sprouting in a stroke brain [44]. In the days to weeks following brain tissue damage, microglia and astrocyte activation shifts its cytokine release profile through the secretion of anti-inflammatory and neurotrophic factors, such as TGF-β, BDNF and NGF [45]. Provision of a scaffold in the tissue cavity can promote host cells to activate endogenous repair processes, such as neurogenesis, and support tissue reconstruction in the stroke damaged brain.

Biomaterials criteria for tissue engineering approach

Biomaterial scaffolds are natural or synthetic 3D polymer networks that provide an appropriate environment for cells to attach, proliferate, and differentiate to facilitate the formation of extracellular matrix (ECM) [46]. It is important to note that the chemical and mechanical properties of a biomaterial determine the fate of transplanted cells, as well as the drug release profile. The extent of cross-linking and rate of degradation are directly affected by the chemical characteristics of the preparation and determine the overall functionality of the biomaterial.

Biocompatible and non-toxic

Biocompatibility of a biomaterial refers to its biological compatibility with the host tissue, as well as all byproducts being non-toxic and avoiding any undesirable effects on the local tissue environment. The long-term biocompatibility of the material with the host brain dictates the effectiveness of the implantation. Most commonly, the number and degree of reactivity of microglia and astrocytes surrounding the biomaterial is used as an indicator of immunorejection [47], or in vivo biocompatibility. The biocompatibility of byproducts from

biomaterial degradation must also be considered, as the byproducts are often bioactive, which can influence the surrounding environment. Another factor affecting the biocompatibility of a material is dependent on the method of polymerization. Photopolymerization of a hydrogel, or crosslinking when exposed to light, can lead to formation of free-radicals which are toxic to the encapsulated cells [48, 49], as well as the host cells, which are already under high oxidative stress after the injury. However, polymerization processes that are dependent on changes in temperature or pH produce little to no free radicals and often polymerize at physiological conditions, making these polymers injectable using a minimally invasive procedure.

Biodegradable

For successful integration into neural networks, it is necessary that the chemical properties of the material allow it to degrade over time. Permanent implants could lead to chronic inflammation and sustained activation of glial cells (i.e. a foreign body response) around the implant [50]. When designing a hydrogel, the degradation rate can have effects on both the functionality of the hydrogel, as well as the host response. For example, a slow degrading hydrogel would be preferred in order to support the transplanted cells to develop their own ECM, extend processes, and integrate into neural networks. However, faster degradation could result in a reduced inflammatory response in vivo. Thus, it is important to develop materials that balance the cell supportive nature of hydrogels, as well as the rate of degradation to avoid any additional immune response.

Injectable hydrogels

Since brain injuries, such as stroke, vary in size and shape, an ideal biomaterial will fill the cavity space and form gel to repopulate the lost tissue. Crosslinking of water soluble polymers produces a hydrogel that has excellent nutrient and oxygen permeability, promoting cell survival inside the scaffold [51]. These biomaterials can be formulated to exist in liquid state at room temperature while forming gels in situ, allowing for minimal invasive delivery through small-gauge needles using MRI guidance [52]. For example, collagen, methylcellulose, and agarose are all temperature sensitive polymers and their gelation rates can easily be controlled by adding other natural polymers such as hyaluronic acid [53]. The high water content of hydrogels makes them very biocompatible and promising candidates for tissue engineering applications. It is an important consideration that large volume of hydrogel injection into the lesion cavity or into the peri-infarct area would cause tissue disruption and increased intracerebral pressure [54, 55], and therefore

an innovating neurosurgical technique that allows for the drainage of ECF should be employed to avoid additional damage [52].

Gelation and retention

Ideally, hydrogel chemistry facilitates the gelation of the material and minimizes any undesirable diffusion away from the injection site. It is important to avoid any reactions with bioactive molecules in the gel and its precursors, since it can significantly lower the cross-linking density and promote interactions with proteins, leading to an inflammatory response [56]. In order to achieve a complete coverage of the lesion cavity, moderate to rapid gelation of the hydrogel is necessary to avoid permeation into the tissue and support the invasion of host cells into the cavity. Furthermore, mixing of the extracellular fluid (ECF) present in the stroke cavity with the injected hydrogel could change the chemical and mechanical properties and hence influence the extent of gelation and retention. Therefore, it is ideal to drain the ECF before or during the hydrogel injection to avoid mixing and increasing the intracerebral pressure. In experiments where partial diffusion into the host tissue is preferred, such as drug delivery, the hydrogel can be formulated with lower concentrations that support diffusion as well as partial retention in the stroke cavity [52].

Biomaterial stiffness and cell invasion

As mentioned above, the chemical properties of the biomaterial affect the mechanical properties – especially the stiffness of material after polymerization. The compressive modulus measures the stiffness of the biomaterial and can be easily varied by changing the percent composition of its monomers [57], or molecular weight of monomer [58, 59]. The stiffness of the prepared hydrogel is known to affect cell proliferation and differentiation in vivo. If the microtubule compression forces determined by scaffold stiffness are outside the sensitivity range of cells, the cells reinforce by increasing or decreasing actin filament building [60]. Rat neural stem cells (NSCs) grown on soft (<1 kPa) hydrogels differentiated primarily into astrocytes and neurons, however cells cultured on stiffer (>7kPa) gels differentiated into oligodendrocytes [61]. In addition, cells cultured on intermediate stiffness (3.5 kPa) showed the most proliferation. Similarly, mesenchymal stem cells (MSCs) responds differently to varying mechanical properties with cells differentiating to neural like cells on soft gels (0.1–1 kPa), osteogenic cells on stiffer (25–40 kPa), and myogenic cells with intermediate stiffness [62]. It is important to note that an ideal biomaterial will closely resemble the mechanical properties of the host tissue to minimize the contact stresses and an aggravated response from immune cells. Indeed, a concentration-dependent cell infiltration was observed after the injection of ECM hydrogel in ischemic rats

[19]. A concentration of 8 mg/mL, with elastic modulus comparable to healthy brain tissue, produced the most significant polarization towards an M2-like macrophage phenotype, as well as the most number of neural progenitors invading the hydrogel. With degradation and loss of cross-links, the compressive modulus of the material decreases and results in loss of mechanical integrity.

Natural biomaterials

Extracellular matrix makes up 20 % of the whole brain tissue volume and plays a role in maintaining key cellular functions [63]. Hydrogels derived from ECM may provide the mechanical properties and signaling molecules to attract host cells into the lesion cavity and obviating the need for exogenous cells [8, 15–17]. The decellularized biomaterial contains ECM proteins (e.g. laminin, fibronectin), myelin and growth factors, including VEGF and fibroblast growth factor-2 [64, 65].

ECM harvested from different organ systems, such as the brain, spinal cord, or urinary bladder influence neural stem cell phenotypic fate and the extent of invasion [66]. While the ECM in the peripheral tissue contains high amounts of collagen, fibronectin and laminin, the adult CNS is mainly composed of glycosaminoglycans and proteoglycans [67, 68]. In a comparison study of ECM derived from brain, spinal cord, and urinary bladder, all ECMs increased the number of cells expressing neurites, but only the brain ECM increased neurite length [69]. Injection of urinary bladder derived ECM hydrogel in rodent stroke brain promoted an acute endogenous repair response, with a significant number of neural progenitors invading the hydrogel [19].

In addition to ECM hydrogels, natural polymers like hyaluronic acid (HA) [70, 71], fibrin [72], HA-methylcellulose (HAMC) [73], chitosan [74, 75], and collagen [76] have been used extensively to deliver cells or molecules in the CNS. Collagen is a popular material used in biomedical applications since it is the most abundant protein and main component of peripheral ECM in mammalian tissues. Collagen hydrogels have been used to encapsulate a variety of stem cell types for tissue engineering applications because of their biocompatibility, mechanical strength and immunogenicity [77]. In a rodent model of cerebral ischemia, encapsulation of NSCs in collagen type I hydrogel showed an increase in cell survival (compared to injection of cells alone), formation of synapsis, and facilitated the functional recovery of neural tissue following injury [46]. Another naturally occurring polysaccharide found in CNS and used for hydrogel formation is hyaluronan. It is known to have anti-inflammatory properties and has been shown to promote cell adhesion and survival [78].

Transplantation of a cross-linked hyaluronan and heparin sulfate hydrogel in a mouse models of ischemia significantly promoted the survival of NPCs, and attenuated infiltration of immune cells into the graft compared with the cells delivered in suspension alone [79].

Synthetic biomaterials

Synthetic biomaterials allow precise control over material properties and degradation rates, slowing controlled release of small molecules or drugs into the surrounding tissue.

The commonly used biomaterials for controlled drug delivery are the polymeric agents polylactide (PL), polyglycolide (PG), and the copolymers of lactide and glycolide (PLGA). PLGA particles are loaded with bioactive molecules and are delivered to the site of injury or embedded in hydrogels to further tune the location and rate of delivery. Synthetic biomaterials, unlike natural materials like collagen and Matrigel, are better chemically defined and biologically inert which reduces the variability and the host immune response. In normal untreated animals, injection of PLGA based microspheres evoked an inflammatory response no greater than just the needle tract [80, 81]. There was a peak in astrocyte activation at 1 week post-transplantation and it diminished as the polymer degraded [80, 82, 83]. Like natural biomaterials, byproducts of synthetic materials can also be bioactive and influence the local microenvironment. In a recent study, application of lactic acid (byproduct of PLGA) on cultured slices of developing mouse cerebral cortex supports oligodendrocyte development and myelination [84]. Nanoparticles and gels made from PGA, PLA and PLGA are primarily used for drug delivery since their degradation rate can be controlled by simply adjusting the PL:PG ratio.

Another synthetic polymer known to resist protein absorption and commonly used in biomaterial applications is poly (ethylene glycol) (PEG). Although cells do not directly attach to PEG hydrogels, it is most often mixed with other polymers like HA or gelatin to support cell attachment and migration. Epi-cortical delivery of PEG modified epidermal growth factor (PEG-EGF) significantly increased tissue penetration and endogenous NSC stimulation compared to unbound EGF [85]. PEG based hydrogels are promising in the fields of drug and cell delivery for many reasons, including controlled drug delivery or degradation rate, nontoxicity and biocompatibility.

Incorporating cells and growth factors

With limited endogenous neurogenesis and capacity to regenerate following injury, delivery of exogenous cells and bioactive molecules to the site of injury has shown to modulate the inflammatory response, stimulate endogenous stem cells, and promote neuroprotection and

plasticity [86]. These transplanted cells can help in the tissue repair process by directly integrating into the host tissue or by secreting factors that promote neurogenesis [87]. Indeed, human NSCs transplanted in the ischemic parenchyma in rats have shown to release factors, such as VEGF and FGF-2, which are effective in stimulating the endogenous neurogenesis [88, 89]. In addition, transplanted NSCs have shown to induce a downregulation of pro-inflammatory cytokines, such as interleukin-1B and tumor necrosis factor alpha, in ischemic mice's brains [90], drastically decreasing the microglia driven inflammatory response [91]. Unfortunately, most of the transplanted cells are lost during the acute inflammatory phase [79, 92], and fail to replace the lost connections. However, hydrogels can provide the necessary microenvironment and survival factors to increase the survival and integration of transplanted cells. Matrigel, a commonly used biomaterial derived from a mouse sarcoma with ECM components collagen, entactin, and laminin has been shown to reduce the infarct size after injury, only when used in combination with transplanting cells [93]. With the ability to protect the cells to improve survival and promote neural cell integration, more studies on cell therapy in combination with protective hydrogels would greatly advance neural tissue engineering.

Incorporation of growth factors and other bioactive molecules in a biomaterial allows researchers to deliver site specific factors with a temporal control over the release profile. The release of trophic factors is not only dependent on the chemical and mechanical properties of the material, but also on the method of encapsulation, such as direct loading or covalent binding. Delivery of VEGF via PLGA microspheres recruits endothelial cells into the graft and promote the development of a local neovascular network [94]. Other neurotrophic factors, such as BDNF, GDND and NGF, have been experimented in treating animal models of stroke and have shown the survival, proliferation, and differentiation of transplanted cells. Vascularization of the injected hydrogel is an important consideration to promote cell invasion and integration with the host tissue. Combination tissue engineering strategies involving cells, growth factors, and biomaterials are currently being pursued as a means to enhance cell survival and integration, as well as local delivery of bioactive molecules in the damaged tissue. Integration of cells and growth factors into the biomaterial as a delivery vehicle can provide physical support for the cells and a sustained drug release profile, thereby avoiding the need for multiple injections for drug delivery.

Exploiting endogenous neurogenesis

The ability to repair the brain after injury is hindered by the inability of neurons to undergo mitosis. Despite the limited repair capacity of the CNS [95], some degree of recovery has been observed in an ischemic brain [21]. Under normal conditions, adult neurogenesis occurs only in two specialized niches, the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus [96, 97]. Indeed, increase in cell proliferation in rodent SVZ [98], and neurogenesis in an adult brain has been reported following stroke [99]. However, neural progenitor cells in an adult brain have difficulty migrating towards the damaged cortex due to the dense white matter tracts [100]. After researching the mechanisms of neuronal repair in a rodent model of stroke, researchers found that less than 1 % of the lost neurons were replaced by endogenous NSCs [101]. In addition, the pool of NSCs are depleted with age [102], which adds another barrier to tissue repair from endogenous stem cells.

Current strategies are looking into pharmacological means to stimulate the proliferation and migration of NSCs into the areas of tissue damage. Activation of endogenous neurogenesis requires administration of key regulators, such as microRNAs, BNDF or GDNF. These neurotrophic factors, administered or released by transplanted cells, have been known to activate specialized signaling pathways that promote the proliferation of NSCs in lateral ventricle and axonal growth and synaptogenesis [103]. Delivery of betacellulin (BTC), a member of EGF family, into the lateral ventricle induced the expansion of NSCs and neuroblasts, as well as neurogenesis in the olfactory and dentate gyrus [104]. Interestingly, recent studies have shown that endogenous repair mechanisms are not limited to neurogenic niches as glial cells including astrocytes, oligodendrocyte precursors, and pericytes can be reactivated following ischemia and differentiate into neurons [105–107].

Conclusions - making it all work

While further development of biomaterials to support the damaged neural environment is still needed, hydrogels use minimally invasive techniques to deliver cells and neurotrophic factors to promote neuroplasticity and angiogenesis, while also promoting the invasion of non-immune cells into the hydrogel. With the ability to control degradation rate and release profile of bioactive drugs, hydrogels provide a promising environment for cell therapy and tissue regeneration.

Exploiting the potential of endogenous neurogenesis to treat brain injury such as stroke has significant advantages over other treatments because it uses the endogenous repair mechanisms to produce functional neurons and participate in the network repair. As the innate capacity to regenerate after injury is limited, stimulating endogenous neurogenesis via exogenous means will only bring us one step closer to our goal of replacing lost neural connections. Combination treatment strategies, including cell and growth factor delivery, stimulating

endogenous neurogenesis and rehabilitation could pave the way forward in promoting neuroplasticity and functional recovery after stroke.

Abbreviations
ATP: Adenosine triphosphate; BBB: Blood brain barrier; BTC: Betacellulin; CBF: Cerebral blood flow; ECF: Extracellular fluid; ECM: Extracellular matrix; GFAP: Glial fibrillary acidic protein; HA: Hyaluronic acid; HAMC: Hyaluronic acid-methylcellulose; IL: Interleukin; IP: Ischemic penumbra; PEG: Polyethylene glycol; PEG-EGF: Polyethylene glycol modified epidermal growth factor; PG: Polyglycolide; PL: Polylactide; PLGA: Copolymers of lactide and glycolide; SGZ: Subgranular zone; SVZ: Subventricular zone; TNF: Tumor necrosis factor

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