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Combination scaffolds of salmon fibrin, hyaluronic acid, and laminin for human neural stem cell and vascular tissue engineering

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ABSTRACT

Human neural stem/progenitor cells (hNSPCs) are good candidates for treating central nervous system (CNS) trauma since they secrete beneficial trophic factors and differentiate into mature CNS cells; however, many cells die after transplantation. This cell death can be ameliorated by inclusion of a biomaterial scaffold, making identification of optimal scaffolds for hNSPCs a critical research focus. We investigated the properties of fibrin-based scaffolds and their effects on hNSPCs and found that fibrin generated from salmon fibrinogen and thrombin stimulates greater hNSPC proliferation than mammalian fibrin. Fibrin scaffolds degrade over the course of a few days *in vivo*, so we sought to develop a novel scaffold that would retain the beneficial properties of fibrin but degrade more slowly to provide longer support for hNSPCs. We found combination scaffolds of salmon fibrin with interpenetrating networks (IPNs) of hyaluronic acid (HA) with and without laminin polymerize more effectively than fibrin alone and generate compliant hydrogels matching the physical properties of brain tissue. Furthermore, combination scaffolds support hNSPC proliferation and differentiation while significantly attenuating the cell-mediated degradation seen with fibrin alone. HNSPCs express two fibrinogen-binding integrins, $\alpha V\beta 1$ and $\alpha 5\beta 1$, and several laminin binding integrins ($\alpha 7\beta 1$, $\alpha 6\beta 1$, $\alpha 3\beta 1$) that can mediate interaction with the scaffold. Lastly, to test the ability of scaffolds to support vascularization, we analyzed human cord blood-derived endothelial cells alone and in co-culture with hNSPCs and found enhanced vessel formation and complexity in co-cultures within combination scaffolds. Overall, combination scaffolds of fibrin, HA, and laminin are excellent biomaterials for hNSPCs.

Statement of Significance

Interest has increased recently in the development of biomaterials as neural stem cell transplantation scaffolds to treat central nervous system (CNS) injury since scaffolds improve survival and integration of transplanted cells. We report here on a novel combination scaffold composed of fibrin, hyaluronic acid, and laminin to support human neural stem/progenitor cell (hNSPC) function. This combined biomaterial scaffold has appropriate physical properties for hNSPCs and the CNS, supports hNSPC proliferation and differentiation, and attenuates rapid cell-mediated scaffold degradation. The hNSPCs and scaffold components synergistically encourage new vessel formation from human endothelial cells. This work marks the first report of a combination scaffold supporting human neural and vascular cells to encourage vasculogenesis, and sets a benchmark for biomaterials to treat CNS injury.

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1. Introduction

Stem cells are an important component of tissue engineering and regenerative medicine approaches since they provide beneficial secreted molecules to stimulate repair and can replace lost cells [1]. CNS neural stem cells are multipotent stem cells capable of self-renewal and differentiation into more committed progenitors that can generate neurons, astrocytes, and oligodendrocytes [2]. Transplanted neural stem and progenitor cells (NSPCs) migrate to areas of injury, actively respond to the microenvironment, secrete neuroprotective compounds, generate differentiated cells, and improve functional recovery [3–7]. However, a significant limitation of stem cell transplantation into the damaged CNS is that most cells die upon injection, with reports of surviving cells ranging from 0% to 30% at 4 weeks post-transplant for non-immortalized human NSPCs (hNSPCs) in rodent models of ischemic stroke [8]. The incorporation of NSPCs into a biocompatible scaffold, which is a 3-dimensional structure that supports tissue formation, can alleviate low cell survival post-transplant. For example, inclusion of a scaffold increases survival of NSPCs transplanted into rodent models of stroke [9] and traumatic brain injury (TBI) [10]. Scaffolds provide a 2-fold increase in mouse NSPC survival 2 weeks post-transplant into the infarct core of a mouse stroke model [9]. Furthermore, scaffolds increase survival of mouse NSPCs in a mouse model of TBI up to 5.6-fold at 8 weeks post-transplant and induce significant improvements in cognitive function [10]. Thus identifying beneficial scaffolds for hNSPCs is critical for realizing their therapeutic potential.

Three-dimensional scaffolds provide stem cells with an appropriate microenvironment and recapitulate the functions of native tissue [11–13]. Thorough scaffold characterization in an *in vitro* setting is essential for optimizing key parameters that support NSPC function. This must be done prior to implementation in animal models of injury in which the *in vivo* niche is quite complex. Critical scaffold attributes for NSPC transplantation into CNS tissue [14] include non-toxic polymerization, biocompatibility with both transplanted NSPCs and host tissue, the ability to be injected as a liquid and polymerize *in situ* to form a tight apposition with the host tissue, and mechanical properties that match that of the CNS. The scaffold must also support vascularization to provide nutrient delivery to cells within the scaffold, have non-toxic degradation by-products and a degradation rate that allows sufficient time for cellular integration. Extracellular matrix (ECM) components such as proteins and polysaccharides are attractive candidates for scaffolds since they are biocompatible, contain sites for cellular adhesion, and provide suitable substrates for stem cell survival, growth, and function.

Fibrin is an ECM protein involved in blood clotting during the coagulation cascade and is non-toxic and biocompatible. Fibrin hydrogels are formed when fibrinogen is cleaved by thrombin to generate fibrin monomers that are covalently crosslinked by Factor XIIIa to create a mesh, which can be degraded by the enzyme plasmin. By varying the concentrations of fibrinogen and thrombin, the mechanical properties and polymerization time of the hydrogel can be modulated [15]. Fibrin contains multiple adhesive sites including RGD sequences that engage integrins on the cell surface. Fibrin has been used as a scaffold for mouse and human NSPCs and as a growth factor delivery vehicle in rodent spinal cord injury models [16–19]. Intriguingly, the source of fibrin can play an integral role in its effectiveness as a scaffold. Salmon fibrin, as opposed to human and bovine fibrin, encourages greater neurite outgrowth of rodent CNS neurons and better resists degradation by cellular proteases [20,21]. Salmon fibrin matches the mechanical characteristics of CNS tissue [20,22] and when used to treat rats with dorsal hemisection spinal cord injuries promotes greater locomotor functional recovery, density of serotonergic fibers caudal to the lesion site, and recovery of bladder function than mammalian fibrin [23]. Salmon fibrin has been developed as a human therapeutic and has passed numerous toxicity and immunogenicity tests [24,25]. Although salmon fibrin is an effective scaffold to treat CNS injury [23], it degrades rapidly *in vivo* (~7 days) and thus is unlikely to provide long-term support for transplanted hNSPCs.

In order to mitigate this rapid degradation, we designed combination scaffolds of fibrin and a material commonly found in the NSPC niche within the brain, hyaluronic acid (HA) [26], which has been shown to persist for at least 2 months when transplanted into the CNS [27,28]. HA is a naturally occurring polysaccharide present in the ECM that is high in the developing brain and in the postnatal brain in regions adjacent to the lateral ventricles where stem cells reside [26,29]. HA has been developed as a biomaterial for NSPC applications [30] including tissue repair after acute ischemic stroke [27,28]. HA scaffolds increase the survival of transplanted mouse NSPCs twofold, promote the differentiation of human induced pluripotent stem cell (iPS)-derived NSPCs into immature neurons, and reduce the host inflammatory response when transplanted into the infarct stroke cavity of a mouse model [9,31]. HA has advantages as a scaffold material but is not always sufficient to promote cell adhesion [32,33], so can be combined with adhesive peptides or another ECM component to provide cell attachment. Thus, combination scaffolds of fibrin and HA may benefit from the cell adhesive properties of fibrin and degradation rate of HA.

Another ECM component beneficial for neural cells that can be incorporated into scaffolds is laminin. Laminin stimulates hNSPC expansion, migration, and differentiation [34] and can be used to functionalize various biomaterials to encourage neural cell adhesion in neural tissue engineering applications [35,36]. Laminin-containing collagen-based scaffolds significantly improve the survival of mouse NSPCs 8 weeks after transplant into the traumatically injured mouse brain and animals treated with laminin-containing scaffolds and NSPCs perform better in behavioral tests than untreated controls [10]. Matrigel scaffolds, which are predominantly collagen and laminin, seeded with embryonic stem cell-derived hNSPCs decrease infarct volume after focal cerebral ischemia in rats compared to cell transplants alone [37]. HA-laminin scaffolds enhance NSPC migration in response to stromal cell-derived factor-1α (SDF-1α) gradients, which arise from injured brain tissue [38]. Interestingly, HA in the scaffolds leads to upregulation of the SDF-1α receptor, CXCR4, on NSPCs while enhanced migration is dependent on both HA and laminin in the scaffolds. Since laminin has beneficial effects on NSPCs and hNSPCs express laminin-binding integrins [34,39], we tested laminin in our combination scaffolds with fibrin and HA.

The biomaterial composition of the scaffold affects its vascularization *in vivo*, which is critical for bringing nutrients to transplanted cells. Crosstalk between endothelial cells and NSPCs also impacts NSPC function and has inspired interest in the *in vivo* neurovascular niche. Endothelial cells promote proliferation and neuronal differentiation of rodent NSPCs in culture and slice models, mediated at least in part through VEGF produced by endothelial cells acting on NSPC VEGF receptors and upregulation of genes in the Notch pathway in NSPCs [40–43]. NSPCs and endothelial cells/vessels are closely associated *in vivo*, creating neurovascular niches that can be mimicked by including both cell types in scaffold constructs [44]. Co-transplantation of mouse NSPCs and either mouse or bovine endothelial cells without a scaffold into rodent stroke models leads to greater numbers of integrated NSPCs, greater NSPC proliferation, more neuronal differentiation, and improved functional recovery [45,46]. A poly (ethylene glycol)-based scaffold containing mouse NSPCs and