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Authors
Nih, Lina Ratiba
Carmichael, Stanley Thomas
Segura, Tatiana

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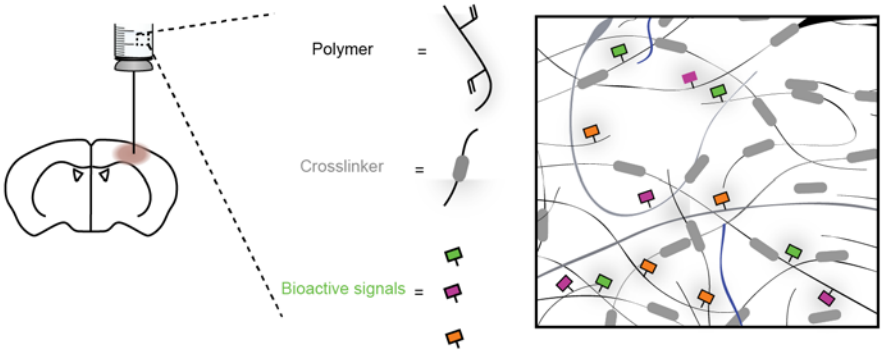
Hydrogels for brain repair after stroke: an emerging treatment option

Lina R. Nih[1], S. Thomas Carmichael[2], Tatiana Segura[1,*]

[1] Chemical and Biomolecular Engineering and [2] Neurology Department, University of California, Los Angeles

* Corresponding author

Abstract
Stroke disability is the only major disease without an effective treatment. The substantial clinical burden of stroke in disabled survivors and the lack of a medical therapy that promotes recovery provide an opportunity to explore the use of biomaterials to promote brain repair after stroke. In situ forming hydrogels can be injected as a liquid and solidify in situ to form a gelatinous solid with similar mechanical properties of the brain. They have been recently explored to generate pro-repair environments within the brain. This review highlights the clinical problem of stroke and discusses recent advances in using in situ forming hydrogels for brain repair. We also discuss recent efforts to use these hydrogels as cell transplantation vehicles to the brain.



Introduction
Stroke is the leading cause of adult disability in the US and the third cause of death worldwide [1]. Ischemic stroke is caused by the occlusion of a vascular structure within the brain and an unsuccessful attempt of the body to establish reperfusion. The subsequent brain injury develops from a complex series of pathological events such as depolarization, inflammation and excitotoxicity [2]. These phenomena dramatically compromise the stability of the Blood-brain barrier (BBB) and activate the release of free radicals and proteases, that not only lead to a local cell death but deepen and extend the injury. Unlike other organs, brain tissue responds to ischemia in a very unique way; while the core of the infarct is immediately and irreversibly damaged, and the associated neurological function is immediately impaired, the boundaries of the core expand to the adjacent tissue over the course of days, spreading apoptotic death to a region that was initially distal from the occluded vessel, and thus perfectly healthy at the stroke onset. Promoting the regeneration of an organ whose wound is dynamic in space and time represents one of the biggest challenges in regenerative medicine, as stroke treatment is now facing very limited therapeutic approaches and an extensive series of unsuccessful clinical trials [3]. This review will explain the general approach, biological problem and discuss the latest hydrogel based therapeutic strategies used to promote brain repair after stroke. For more comprehensive reviews for biomaterial approaches in the CNS, please see [4].

1. Post-stroke endogenous repair mechanisms and targeted therapies
Stroke patients show some degree of recovery over time, independently of the treatment chosen. Below we present the main endogenous repair mechanisms activated after stroke, and how a tissue engineering approach can be exploited to overcome some of the current therapies limitations.

1.1 Inflammation-induced cavity
The massive cell death that occurs following stroke results in the activation of local inflammatory cells or microglia [5], the expression and activation of extracellular matrix (ECM) degradating enzymes (e.g. matrix metalloproteinases, hyaluronidases, serine proteases, etc.) and the loss of mechanical integrity of the

damaged tissue [6]. In order to limit this matrix degradation to the boundaries of the stroke, astrocytes undergo an extensive morphology remodeling and extend processes around the lesion to form a scar [7] that compartmentalize the degraded tissue within a physical empty cavity. Thus, cells attempting to infiltrate the stroke cavity are faced with a physical barrier and a landscape that is not amenable to migration because it is much softer and less elastic than normal brain [6] and is likely lacking of the natural ECM integrin ligands, fibers and structural features necessary for cell migration. A reduced scar thickness was shown to be associated with improved neurological outcome [8].

1.2 Neurogenesis

A major advance within the past decade has been the discovery of ongoing adult neurogenesis, continuous proliferation and maintenance of neural progenitor cells (NPCs) along the ventricles and in the hippocampus [9]. Under normal conditions, these newborn neurons migrate toward the hippocampus and the olfactory bulb. However, stroke damage was shown to increase NPCs proliferation and re-route them towards the damaged site [9]. Whether these NPCs fully differentiate and contribute to stroke patient's functional recovery remains uncertain as the majority of newborn neuroblasts die prematurely within their migratory path.

1.3 Local angiogenesis

Experimental and clinical studies show that enhanced vessel formation and restored perfusion in the ischemic border correlate with improved long-term functional recovery and longer survival of stroke patients [10]. Measurements of the vascular endothelial growth factor (VEGF) levels in both serum and brain tissue of stroke patients showed a positive correlation between the severity of damage and the concentration of the growth factor, suggesting its involvement in the subsequent repair process resulting in recovery [11]. Interestingly, post-stroke neurogenesis and angiogenesis are tightly linked. Indeed, the specific inhibition of vascular growth worsens both neurogenesis and the neurological deficit, leading the path for a new approach based on the administration of VEGF to promote brain tissue regeneration [12].

2. Current therapeutic strategies

The only FDA-approved treatments in stroke focus on acute brain injury by promoting reperfusion. These approaches include direct clot lysis with tissue plasminogen activator (tPA), and endovascular stent/retrievers, which have shown substantial clinical trial success [13] and are likely to be soon approved for use. There is no medical therapy that promotes repair and recovery in this disease. Physical medicine approaches, such as physical, occupation or speech therapy, have a role in promoting a limited recovery after stroke [14].

Stem or progenitor cell transplantation after stroke promotes recovery in pre-clinical models. NPC transplant after stroke has been limited by poor survival of the transplant. This occurs because of immunological attack, and also from abrupt withdrawal of growth factor and adhesive support in placing progenitor cells in a suspended form into a post-ischemic brain [15]. Transplanted NPCs that do survive often remain in an undifferentiated state [15].

The delivery of soluble VEGF has been unsuccessful, as the early systemic or intracerebral administration of VEGF after stroke increased BBB opening and edema, and formed disorganized and immature vasculature while its antagonist reduced the injury [16].

The substantial clinical burden of stroke in disabled survivors and the lack of a medical therapy that promotes recovery provide an opportunity to explore the use in situ tissue regeneration strategies to promote brain repair after stroke.

3. General approach to use hydrogels to guide brain repair after stroke

Classical tissue engineering strategies involve the *ex vivo* generation of engineered organs that can be implanted to substitute the lost tissue. However, this approach is not well suited for brain repair because it requires the invasive implantation of the tissue construct and it is unclear if functional brain tissue could be engineered/produced ex vivo. *In situ* tissue regeneration aims to completely bypass the *ex vivo* generation of the engineered organ, by implanting a scaffold directly at the site of injury in order to stimulate endogenous tissue repair through the use of local or transplanted progenitors. Although early materials for brain repair utilized implantable materials [17,18], recent efforts have focused on engineered injectable hydrogel scaffolds that can be directly transplanted within the stroke cavity for a minimally invasive procedure [19], can be

crosslinked to match the mechanical properties of the normal brain, and can be used as local drug delivery depots (Figure 1). Hydrogels are formed by reducing the mobility of water-swollen polymers via the introduction of physical and/or covalent bonds between polymer chains, creating a network. Depending on the polymer chain length and its tendency to coil and percolate, the crosslinking point and network mesh size can be modulated, which in turn modulates nutrient diffusion and cell motility.

In situ forming (injectable) hydrogel materials offer a unique platform to bioengineer pro-repair environments directly at the stroke site. Hydrogels can promote repair through providing structural support to the surrounding tissue to minimize secondary cell death and manage the inflammatory response. They can also effectively bypass the BBB by local injection of drug-loaded hydrogels, which can encourage cells from the surrounding parenchyma to infiltrate the scaffold and promote local regeneration. Injectable hydrogels can also serve as a cell transplantation vehicle to deliver NPCs. However, access to the brain and the skull require invasive delivery, thus the direct delivery of cells into the brain after stroke in conjunction with a stabilizing or pro-repair biomaterials implant must be controlled so that tissue adjacent to the infarct is not damaged [15]. Although in pre-clinical models the location of the stroke and the injection volume can be controlled such that hydrogel injections are possible without harmful effects, this will not be the case for translation. Thus, intracerebral delivered hydrogels must not swell significantly to avoid further brain damage and ideally injection would be guided using a non-invasive imaging approach [20]. The Modo laboratory has successfully utilized MRI guided hydrogel injection and draining the brain simultaneously as the hydrogel was injected to prevent intracranial pressure buildup [21].

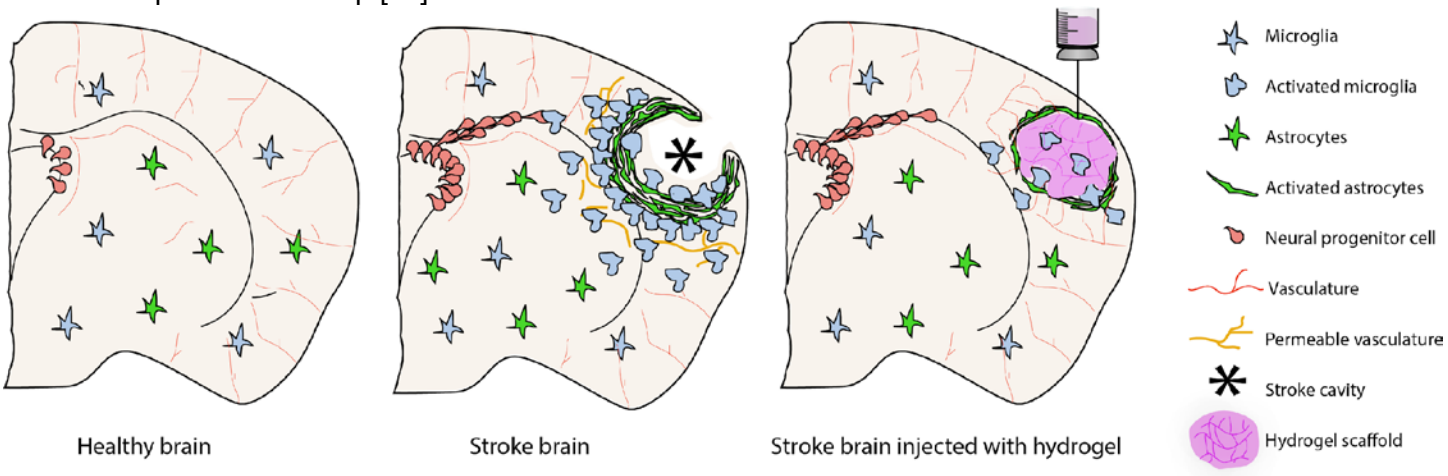


Figure 1: Schematic illustration of a coronal brain section and the major physiopathological events occurring after an ischemic stroke. In order to protect the healthy parenchyma from nearby lesion area, star-shaped glial cells, astrocytes, elongate cytosolic processes to surround the damaged site, forming the astrocytic scar. The long-term persisting peri-lesion scar is known to act as a physical barrier to tissue regeneration by blocking the way to axonal, vascular and neuronal infiltration. After the initial cell death in stroke, the activation and recruitment of microphage-like microglia allows for the clearance of debris in the lesion, leaving a compartmentalized cavity that can accept a large volume transplant without damaging further the surrounding healthy parenchyma. This stroke cavity is situated directly adjacent to the region of the brain that undergoes the most substantial repair and recovery, the peri-infarct tissue, meaning that any therapeutic delivered to the cavity will have direct access to the tissue target for repair and recovery.

4. Biomaterial-based targeted therapy

4.1 Gel implantation for post-stroke brain tissue inflammation

The first and most important step in the bioengineering of materials for brain repair after stroke is the development of a scaffold that contains the necessary mechanical [22-24], topographical, and integrin binding features to allow, promote and guide cellular infiltration and axonal growth into a stroke cavity. One advantage of hydrogel biomaterials is that they show anti-inflammatory properties after inflammation if the mechanics match that of the surrounding tissue [25]. In situ formation of empty hyaluronic acid (HA)/peptide hydrogels

directly at the stroke cavity showed differential macrophage activation depending on matrix stiffness: stiff hydrogels with a bulk storage modulus of 1300Pa showed an increased macrophage density at the tissue/material interface compared to a softer gel [26], showing anti-inflammatory properties of materials that match the brain mechanical properties. Similarly, a functionalized self-assembling peptide hydrogel was shown to reduce the formation of the glial scar two weeks after stroke [27]. Hydrogels can then be loaded with anti-inflammatory drugs such as osteopondin to further limit inflammation. Gelatin microspheres loaded with osteopondin were injected into the striatum of stroked mice. Delivery from the microspheres showed longer lasting levels of osteopondin, decreased inflammation, and increased neuroprotection after stroke [28] (Figure 2).

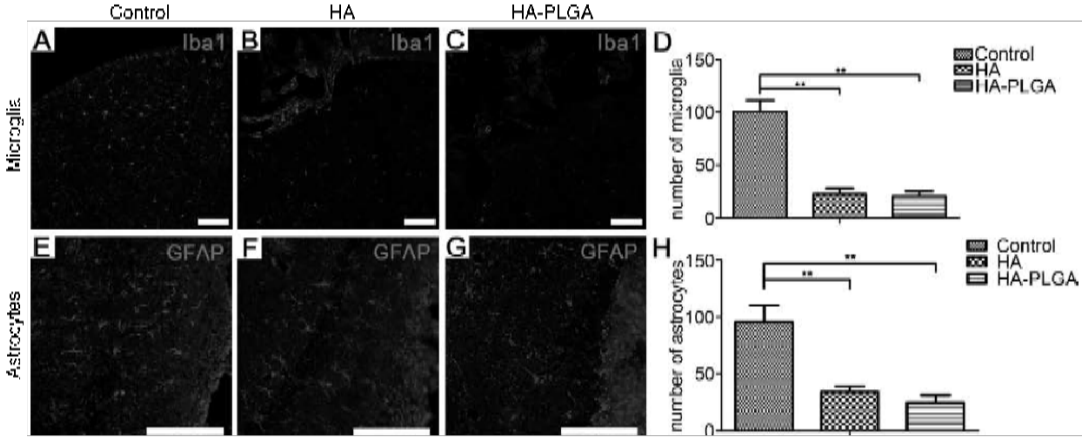
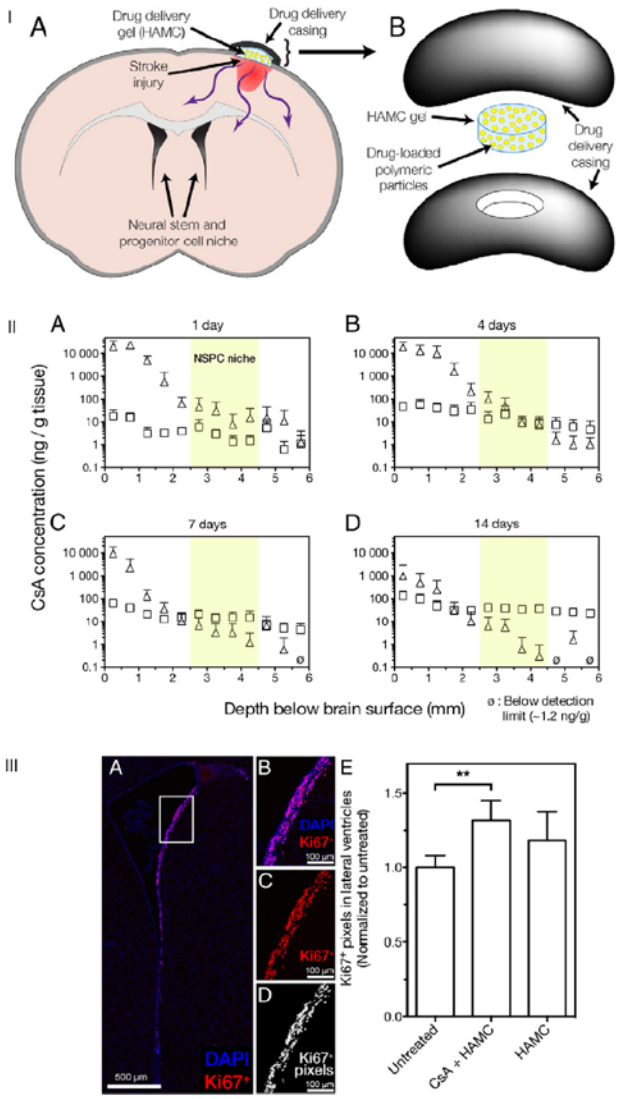


Figure 2: Fluorescent microscopy showing brain inflammation (reactive microglia, Iba1 - Ionized calcium-binding adapter molecule 1 staining) and the astrocytic scar (GFAP - Glial Fibrillary Acid Protein staining) in a mouse stroked brain transplanted with HA and HA–PLGA (poly(lactic-co-glycolic acid), compared with a negative control brain with no implant. The results show a significantly reduced number of astrocytes and microglia in both transplanted groups compared with the control (**$p < 0.01$). Scale bar: 100 μm. Adapted with permission from [29].

4.2 Gel encapsulation of neural stem cell delivery and neuroprotection

Engineering strategies that can further encourage the migration, survival and differentiation of NPCs by promoting a stem-cell niche like environment provides an avenue to rebuild a functional neuronal network. The Shoichet lab has engineered an injectable physical hydrogel (no covalent bonds) composed of a hyaluronan/methylcellulose (HAMC) blend. This hydrogel is applied epi-cortically through injection (Figure 3) and has been used to achieve sustained and sequential delivery of erythopoietin [30] [30,31], epidermal growth factor (EGF) [32] and most recently cyclosporine A (CsA) [33]. Encapsulated EGF released from this system increased NPC proliferation in uninjured and stroke-injured brains, while modifying EGF with polyethylene glycol (PEG) significantly enhanced protein stability, diffusion distance, and in vivo bioactivity. Encapsulated EPO release resulted in an attenuated inflammatory response, reduced stroke cavity size, and increased neurogenesis [31]. Sequential delivery of EGF-PEG and EPO by encapsulation of these factors in PLGA micro/nanoparticles and subsequent delivery within the hyaluronan/methyl cellulose (HAMC) blend reduced inflammation, significantly improved neurogenesis, and minimized tissue damage compared to intracerebroventricular infusion. CsA delivery showed increased drug concentration in the endogenous NPC niche compared to mini-pump delivery of the same drug especially at later time points [33]. This result indicates that local drug delivery from injectable hydrogel formulations can outperform traditional bolus delivery in the brain.

Figure 3: Schematic illustration of the injured-brain with the drug delivery HAMC scaffold device to achieve epi-cortical sustained local delivery to the brain (IA). Drug delivery system in expanded view shows that HAMC is held in place by both gelation and a casing comprised of polycarbonate discs (IB). CsA delivery from HAMC-PLGA composite (Δ) provides sustained release to the stroke injured rat brain. CsA systemically delivered with



a subcutaneous while osmotic minipump (□) diffuses across the BBB into the brain at similar levels throughout the depths examined. CsA penetration and spatial distribution in the ipsilateral hemisphere of stroke-injured rats was examined using high performance liquid chromatography with tandem mass spectometry (HPLC–MS/MS) after delivery for (IIA) 1 day, (IIB) 4 days, [34] 7 days and (IID) 14 days (mean + standard deviation reported). CsA diffuses from HAMC to the NPC niche located 2.5 to 4.5 mm from the brain surface (IIA–D, highlighted in yellow). Epi-cortical delivery of CsA from HAMC increased the amount of Ki67+ proliferating cells in the lateral ventricles of stroke-injured rats. Representative images of Ki67, a marker for cell proliferation + staining of cells along the lateral ventricles (IIIA, IIIB) with (IIIC) and without a nucleus staining (Dapi). (IIID) The number of Ki67+ pixels along the dorsolateral ventricle wall was used to quantify the number of Ki67+ cells in both cerebral hemispheres (mean + standard deviation reported). Only treatment with CsA + HAMC significantly increased the Ki67+ signal in the ventricles ($p = 0.006$ vs. untreated). Adapted with permission from [33].

4.3 Tissue engineering strategies to enhance transplanted cell survival and engraftment

Materials to promote survival must be biocompatible but also bioresorbable to allow transplanted cells to degrade the biomaterials as they spread within the gel, form a network and migrate towards the peri-ischemic area where they can connect an existing network. Indeed, NPCs transplanted within a commercially available HA/heparin/collagen hydrogel into the infarct cavity 7 days after stroke promoted the survival of NPCs and also diminished inflammatory infiltration of the graft [35]. Similarly, transplantation of NPC within type I collagen scaffolds into the ischemic injury facilitated the structural recovery of the neural tissue and improved neurological function [36]. The same result was shown with NPCs encapsulated in a functionalized self-assembling peptide hydrogel [27].

4.4 Cell instructive biomaterials for transplanted cells differentiation

In addition to promoting transplanted cell survival, the delivery of NPCs within a hydrogel matrix provides the opportunity to guide transplanted cell differentiation. A powerful method to dictate cellular phenotype and differentiation is through the incorporation of ECM derived peptides such as the fibronectin-derived peptide RGD and the laminin-derived peptides IKVAV and YIGSR. These peptides are generally added at a 1:1:1 molar ratio. However, through using design of experiments [37] multifactorial optimization, we found that the optimal concentration for NPC cell differentiation to immature neurons is 100 µM RGD:300 µM IKVAV:48 µM YIGS [38] (Figure 4). Thus, the design of cell-material interaction to promote brain repair might not be as simple as adding RGD only or equimolar peptide concentrations. Furthermore, the over-expression of integrins via viral transduction has been shown to enhance the regenerative capacity of adult neurons [39]. Thus, material-inducing over-expression of selected integrin receptors may lead to enhance regenerative capacity of infiltrating neurons. NPC transplantation on PLGA release VEGF microparticles to the stroke cavity showed increased NPC differentiation towards astrocytes and neurons and an enhanced revascularization of the stroke area [40].

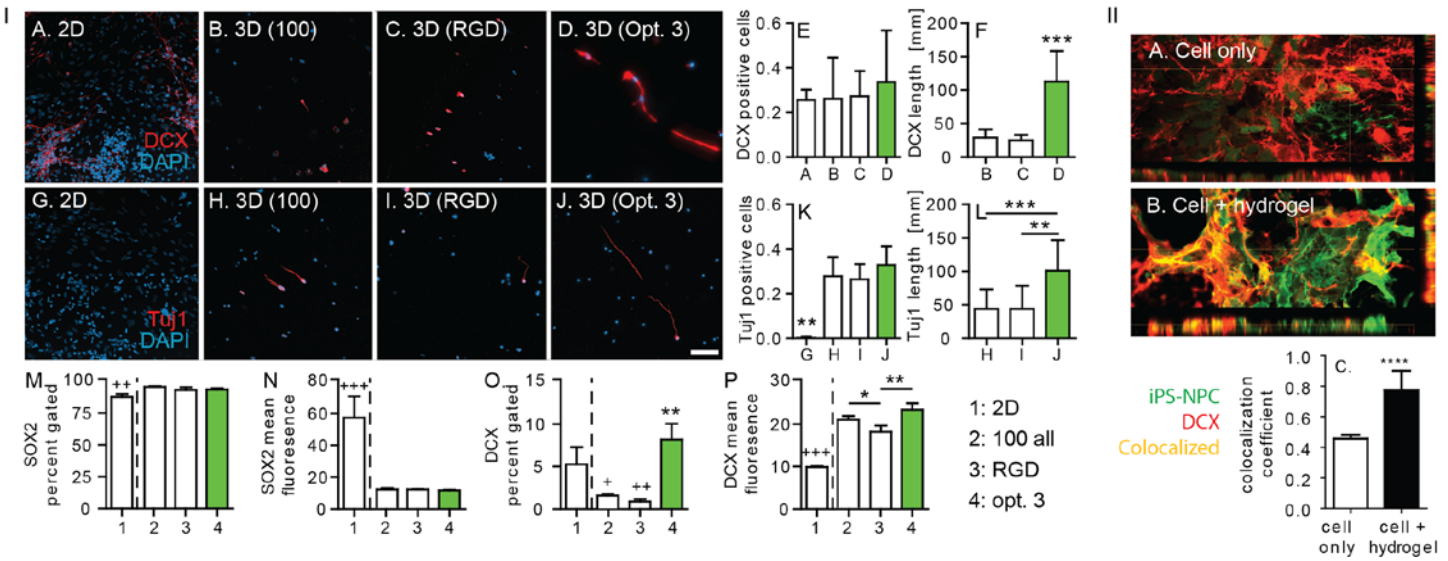


Figure 4: (I) iPS-NPCs cultured for 1 week in A) 2-D, and B–D) hydrogels were stained for Doublecortin (Dcx) and DAPI. Number of E) Dcx-positive cells and F) their axonal length were quantified. Cells were also stained and quantified for tuj1, a marker of mature neuron (G–J, K) and (L) their axonal length quantified. Flow cytometry data for M,N) SOX2, a marker of immature NPC and O,P) Dcx, indicate that the 3D HA hydrogels promote differentiation of the iPS-NPCs. *'s: difference between three dimensional samples. +'s: difference between 3D and 2D sample. Scale bar = 100 μm. */+: $p < 0.5$, **/++: $p < 0.01$, ***/+++: $p < 0.001$. (II) 3-dimensional reconstruction of (A) cell only and (B) cell + hydrogel sections stained for GFP-labeled transplanted cells and Dcx. (C) Colocalization analysis shows that the majority of Dcx positive signal seen in cell + hydrogel condition is from transplanted cell differentiation. Adapted from [38].

4.5 Nanotechnology-based Vascular growth factor delivery

As mentioned previously, both systemic and intracerebral administration of VEGF have been unsuccessful and associated with severe side effects such as edema and hemorrhage. Interestingly, when administered repeatedly, delayed or encapsulated in a hydrogel, VEGF delivery consistently improved the recovery and reduced the injury, with an overall better effect when administered locally than systemically [16]. Therefore, maintaining elevated tissue levels of VEGF in the stroke site for prolonged periods of time, with controllable spatial distribution, dosage and duration of exposure via its encapsulation within transplantable hydrogel might be the ideal solution to overcome the main limitations of poor penetration across the BBB, the clinically unviable option of repeated local injections, and the VEGF short half-life time. A perfect example of this is illustrated by a composite non-injectable HA scaffold containing PLGA loaded with VEGF and angiopotein-1 microparticles, and conjugated with an antibody against NOGO, an axonal growth inhibitor, resulting in an improved vascularization and recovery by a controlled release of the growth factors [29]. Our group recently demonstrated the sustained delivery of VEGF from an in situ forming HA gel crosslinked with an MMP-sensitive crosslinker and containing RGF motifs, directly at the stroke cavity. VEGF is encapsulated within protease labile, water-soluble nanocapsules formed through in situ radical polymerization of acrylate-modified peptides (release rate is controlled through mixing L and D crosslinking peptides). We demonstrated that a controlled release in vivo is associated with improved vascularization within the stroke peri-infarct and infarct region [41].

Conclusions and future perspectives

Stroke is a medical emergency representing the most common cause of severe and long-term disability in adults. However, despite the widespread practice and proven benefit of reperfusion therapies, the majority of patients are left with a long-lasting neurological impairment. Over the past few years, new therapeutic strategies aimed at enhancing endogenous repair mechanisms of the brain, such as post-stroke neurogenesis

and angiogenesis, however their clinical translation has failed, mainly because of the short half-life and systemic effects of injected growth factors [2] and poor survival of transplanted cells. The lack of a successful medical therapy that promotes long-term recovery represents a tremendous clinical and economic burden, urging the need to search for a medical solution outside the confines of conventional treatments practiced in neurology. Recent advances in tissue engineering have developed injectable biomaterial that can serve as a protective vehicle for both cells and trophic factors with distinct advantages compared with simple injection of either alone. The overall premise of in situ tissue regeneration is that by implanting a scaffold at the site of injury or disease, one could engineer a reparative niche that would lead to local tissue repair. Polymer hydrogels show numerous advantages as a wide variety of chemical, mechanical and spatial cues can be incorporated to adapt to the host tissue and to the encapsulated vehicle.

However, the biology of brain repair is not completely understood, and thus the repair programs to be activated as well as the rate of matrix degradation and host cell infiltration must be revisited, as new information is available. Furthermore, although tissue engineered strategies have been around for at least two decades, they have not been consistently applied to brain repair after stroke. Thus, there are a number of existing strategies that could be applied to the brain such as the incorporation of an open pore structure, topographical cues, and using hydrogel mechanics to guide differentiation. Although an open pore structure has been shown to be superior for tissue infiltration and repair in other organs [42,43] porous hydrogels have not been used for brain repair probably because these materials are typically non-injectable. However, two approaches to generate injectable porous hydrogels have been recently published [44,45]. Both manuscript report superior tissue repair and reduced inflammation due to the porous nature of the hydrogel. In addition, topographical guidance cues have been extensively studied to guide spinal cord axonal sprouting [46], but have not been used to guide brain axonal sprouting. Thus the introduction of porosity and topographical guidance cues offer exciting new possibilities for brain repair. Last, mechanical guidance cues have been shown to be a powerful differentiation cue to neural progenitor cells [24]. However, the use of mechanics has not been exploited in vivo to guide differentiation of transplanted or local progenitors, likely because soft matrices (needed to guide neuronal differentiation) tend to degrade faster than desired. Thus, approaches to decouple matrix mechanicals from matrix stability are needed to fully exploit mechanical guidance cues. A number of new studies report on hydrogels that contain both physical and covalent bonds, which are soft and have long lasting mechanical support because the physical bonds can break and reform as the cells grow or the tissue infiltrate [47-49]. Physical hydrogels with covalent stabilization were used as a stem cell transplantation to the stroke cavity and shown to promote stem cell survival and angiogenesis without the delivery of angiogenic factors [50]. Along with physical guidance the delivery of trophic factors maybe needed to further encourage axonal sprouting functional connections. BDNF for example is known to be implicated in modulating synaptic plasticity [51] and was shown over a decade ago to be unsuccessful when administered alone [52], but enhanced axonal regrowth in damaged tissue after stroke [53] when delivered within a methacrylamide (HPMA) hydrogel implant.

Research Highlights

- Stroke is the leading cause of adult disability
- In situ forming hydrogels can be injected directly into the stroke cavity
- In situ forming hydrogels can be used to construct pro-repair environments in the brain after stroke
- Drug-loaded in situ forming hydrogels can be used to bypass the BBB for the delivery of drugs to the brain

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