

Development of New Stem Cell-Seeded Hydrogels to Support the Recovery of Brain Structure and Function after Stroke

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ABSTRACT

Cerebral ischemia is a devastating of the brain without regenerative treatment options, demanding a vigorous search for new therapeutic strategies. Despite the initial hope that cell-based therapies may stimulate restorative processes in the ischemic brain, it is now recognized that aging processes may promote generate an unfavorable environment for such treatments. By this project we take advantage of our previous experience on stroke therapies and aim at developing of a novel stem cell-seeded hydrogel to support the recovery of brain structure and function after stroke by exploiting new developments in the stem cell biology and nourishing hydrogels for cell culture of neuronal cells. Human stroke data suggests that the cortical ischemic core but not the penumbra is a determinant of clinical outcomes after acute ischemic stroke. Therefore any therapeutic delivered to the cavity will have direct access to the tissue target for repair and recovery. Recent advances in tissue engineering have developed injectable hydrogels that can provide both a mechanical support and trophic factors for neuronal precursor cells (NPCs). Since stroke affects mostly the elderly, it is highly desirable and clinically important to test the efficacy of cell therapies in aged brain microenvironments. Recently new technologies to promote regeneration in the damaged brain area have been developed by embedding stem cells in nourishing hydrogels, thereby recreating a neurovascular niche by recruitment of neural precursor cells and microvascular cells. Through this novel experimental technique ones expect a significant improvement in tissue integrity and functional restoration after stroke. Given the overwhelming importance of stroke therapy for both patients and society, this approach, if successful, will be a breakthrough in the field.

Key words: stroke; therapy; stem cells; hydrogels; recovery

INTRODUCTION

Stroke has limited treatment options, demanding a vigorous search for new therapeutic strategies. Despite the initial hope that cell-based therapies may stimulate restorative processes in the ischemic brain, it is now recognized that aging processes may promote generate an unfavourable environment for such treatments. Old age is associated with an enhanced susceptibility to stroke and aged animals, recover poorly from brain injuries compared to young. Since stroke affects mostly the elderly, it is highly desirable and clinically important to test the efficacy of cell therapies in aged brain microenvironments. We have shown that the aged rat brain is not refractory to cell-based therapy as previously thought, and that it also supports plasticity and remodelling. Yet, important differences exist in the aged compared with

young brain, i.e., the accelerated progression of ischemic injury to brain infarction, the reduced rate of endogenous neurogenesis and the delayed initiation of neurological-

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-recovery. These age-related aspects should be carefully considered in the clinical translation of restorative therapies.

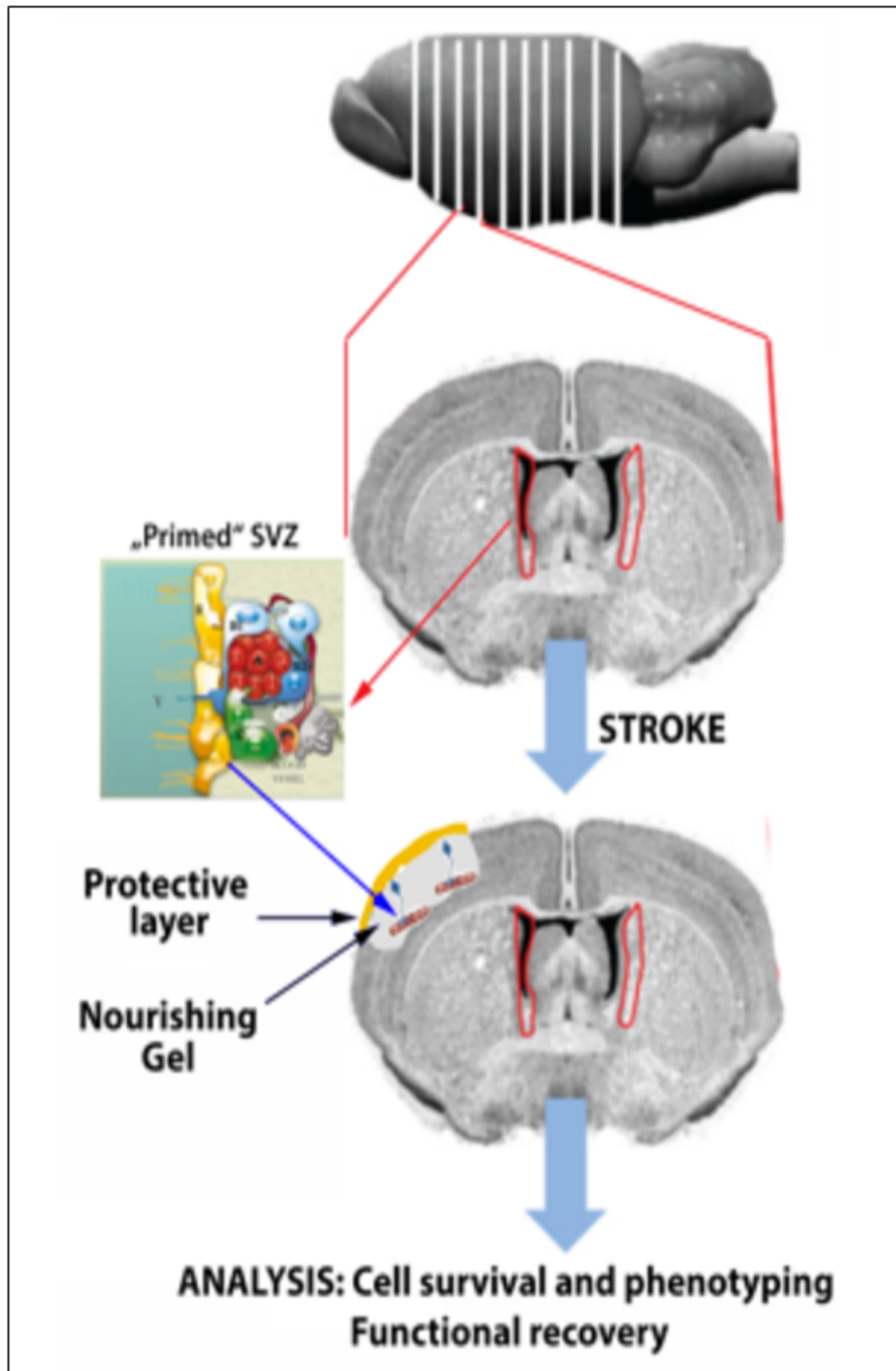


Figure 1. Schematic illustration of the stem cells seeded hydrogels for stroke therapy.

We found that at genetic and cellular level there are significant differences in behavioral, cytological and genomics responses to injury in old animals as compared with the young ones [Popa-Wagner A et al., 2011; Buga AM et al., 2013]. Behaviorally, the aged rats have the capacity to recover after cortical infarcts albeit to a lower extent than the younger counterparts. Similarly, the increased vulnerability of the aged brain to stroke, together with a decreased interhemisphere synchrony after stroke, assessed by different experimental methods (MRI, fMRI, in vivo microscopy, EEG) leads to unfavorable recovery of physical and cognitive functions in aged people and may have a prognostic value for the recovery of stroke patients. Furthermore, in elderly, comorbidities like diabetes or arterial hypertension are associated with higher risk of stroke, increased mortality and disability, and poorer functional status and quality of life. Aging brain reacts strongly to ischemia-reperfusion injury with an early inflammatory response. The process of cellular senescence can be an important additional contributor to chronic post-stroke by creating a “primed” inflammatory environment in the brain. Overall, these proinflammatory reactions promote early scar formation associated with tissue fibrosis and reduce functional recovery. A better understanding of molecular factors and signaling pathways underlying the contribution of comorbidities to stroke-induced pathological sequelae, may be translated into successful treatment or prevention therapies for age-associated diseases which would improve lifespan and quality of life [Popa-Wagner A et al., 1998; Popa-Wagner A et al., 2006; Popa-Wagner A et al., 2011; Buga AM et al., 2013; Di Napoli M et al., 2012; Badan I et al., 2003; Buchhold B et al., 2007].

After cortical stroke, a cavity and a bordering scar to the perinfarct, develop. Contrary to a commonly held view that the scar impairs neural recovery and repair, we have shown that the poststroke scar is actually vascularized fibrous tissue [Balseanu AT et al., 2014]. This finding suggests that astrocytic scar formation is not a principal obstacle to the regrowth of injured axons across severe CNS lesions, and that scar-forming astrocytes may actually support the regeneration of appropriately stimulated CNS axons [Buga AM et al., 2014; Buga AM et al., 2015; Anderson MA et al., 2016]. Moreover, any therapeutic delivered to the cavity will have direct access to the tissue target for repair and recovery.

Many vascular endothelial cells and neurovascular structures in the core survive from the ischemic insult and regenerative activities such as proliferation of endothelial cells and formation/invasion of neural progenitor cells takes place in the core many days after stroke. As a result, extensive neurovascular networks are established in the ischemic core 14 days after stroke [Popa-Wagner et al., 2007; Buga AM et al., 2014]. Although the surviving cells in the core after stroke are few and the neurovascular structures may be imperfect or immature, they could provide a minimum but

vital infrastructure for possible regeneration from endogenous mechanisms. For regenerative therapies using exogenous stem cells and neural progenitors, our data suggest that the microenvironment of ischemic core several days after stroke provides certain cellular and strong trophic supports for cells to survive while the remaining and regenerating neurovascular infrastructure may be utilized for repair of damaged neural networks.

Attractive therapeutic strategies stimulating and finally enhancing the natural post-stroke regeneration process include methods of training such as physio- or rehabilitative therapy or methods of cellular therapy [Hermann DM et al., 2012; Honmou O et al., 2012; Popa-Wagner A et al., 2014]. Stroke induces a specific remodeling of the brain vasculature. Using an aged rat model of stroke, we previously found that at two weeks after stroke the microvascular density was reduced in aged rats as compared to young animals on a background of persistent upregulation of genes coding for matrix proteases and inflammatory mediators. However, beyond the inhibitory fibrotic scar, in a region made of soft tissue that we dubbed “islet of regeneration”, the vascular density was similar in the two age groups. Unlike in rats, the post-stroke angiogenesis in human patients is vigorous at one week post-stroke, and correlates well with the post-stroke survival time. By comparative transcriptomics of angiogenesis we identified 36 new stroke-related genes some of which may be used as new therapeutic targets that may help redress the dysregulation of *angiogenesis* in the infarcted area of aged brain. We also found that the aged human brain is capable of mounting a vigorous angiogenic response after stroke, which most likely reflects the remaining brain plasticity of the aged brain [Buga AM et al., 2014; Raluca Elena Sandu et al., 2016].

We were also concerned with identifying differences in gene expression between the young and the aged brain after a lesion such as stroke. To this end, we employed proteomics and the Affymetrix platform to analyze the whole-gene transcriptome following temporary ligation of the middle cerebral artery in aged and young rats. The correspondence, heat map, and dendrogram analyses independently suggest a differential, age-group-specific behaviour of major gene clusters after stroke. Overall, the pattern of gene expression strongly suggests that the response of the aged rat brain is qualitatively rather than quantitatively different from the young, i.e. the total number of regulated genes is comparable in the two age groups, but the aged rats had great difficulty in mounting a timely response to stroke. Our study indicates that four genes related to neuropathic syndrome, stress, anxiety disorders and depression (*Acvr1c*, *Cort*, *Htr2b* and *Pnoc*) may have impaired response to stroke in aged rats. New therapeutic options in aged rats may also include *Calcr1*, *Cyp11b1*, *Prcp*, *Cebpa*, *Cfd*, *Gpnmb*, *Fcgr2b*, *Fcgr3a*, *Tnfrsf26*, *Adam 17* and *Mmp14*. An unexpected target is the enzyme 3-hydroxy-3-methylglutaryl-Coenzyme A synthase

1 in aged rats, a key enzyme in the cholesterol synthesis pathway. Post-stroke axonal growth was compromised in both age groups. Our results suggest that a multi-stage, multimodal treatment in aged animals may be more likely to produce positive results. Such a therapeutic approach should be focused on tissue restoration but should also address other aspects of patient post-stroke therapy such as neuropathic syndrome, stress, anxiety disorders, depression, neurotransmission and blood pressure [Junker H et al., 2007; Buga AM et al., 2008; Buga AM et al., 2012; Joseph C et al., 2012; Buga AM et al., 2014].

Although the surviving cells in the core after stroke are few and the neurovascular structures may be imperfect or immature, they could provide a minimum but vital infrastructure for possible regeneration from endogenous mechanisms. For regenerative therapies using exogenous stem cells and neural progenitors, our data suggest that the microenvironment of ischemic core several days after stroke provides certain cellular and strong trophic supports for cells to survive while the remaining and regenerating neurovascular infrastructure may be utilized for repair of damaged neural networks.

Attractive therapeutic strategies to enhance post-stroke recovery of aged brains include methods of cellular therapy that can enhance the endogenous restorative mechanisms of the injured brain. Since stroke afflicts mostly the elderly, it is highly desirable to test the efficacy of cell therapy in the microenvironment of aged brains that is generally refractory to regeneration. In particular, stem cells from the bone marrow allow an autologous transplantation approach that can be translated in the near future to the clinical practice. Such a bone marrow-derived therapy includes the grafting of stem cells as well as the delayed induction of endogenous stem cell mobilisation and homing by the stem cell mobiliser Granulocyte-colony Stimulating Factor (G-CSF). In previous work, we tested the hypothesis that grafting of bone marrow-derived pre-differentiated mesenchymal cells (BM MSCs) in G-CSF-treated animals improves the long-term functional outcome in aged rodents. To this end, G-CSF alone (50 µg/kg) or in combination with a single dose (10^6 cells) of rat BM MSCs were administered intravenously to Sprague-Dawley rats at six hour safter transient occlusion (90 min) of the middle cerebral artery. Infarct volume was measured by MRI at 3 and 48 days post-stroke and additionally by immunohistochemistry at day 56. Functional recovery was tested during the entire post-stroke survival period of 56 days. Daily treatment for post-stroke aged rats with G-CSF led to a robust and consistent improvement of neurological function after 28 days. The combination therapy also led to robust angiogenesis in the formerly infarct core and beyond in the “islet of regeneration”. However, G-CSF + BM MSCs may not impact at all on the spatial reference-memory task or infarct volume and therefore did not further improve the post-stroke recovery. We suggest that in a real clinical practice involving older

post-stroke patients, successful regenerative therapies would have to be carried out for a much longer time [Balseanu AT et al., 2014; Buga AM et al., 2015].

Recent advances in tissue engineering have produced hydrogels have been designed to promote stem cell survival, minimize wound scar formation, and enhance stem cell engraftment [Potter et al., 2008; Chai et al., 2007]. Hydrogels for CNS applications are easily transplanted into the adult brain without damage and support survival and differentiation of stem/progenitor cells in vitro and in vivo. Further, very important, hyaluronan gels have mechanical properties similar to brain tissue and do not promote local scarring or tissue reaction These gels influence neural differentiation and allow neuronal sprouting and ingrowth into the gel [Van Wie BJ et al., 2007; Zhong J et al., 2010; Nih et al., 2016].

Finally, the stroke cavity containing the embedded SVZ will be sealed by a protective layer containing microvascular endothelial cells and dermal fibroblasts consisting of a collagen-GAG, three-dimensional matrix colonized by human dermal fibroblasts [Froget S et al., 2003].

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