Journal of Ageing and Restorative Medicine
JARM, 1(1): 13-18
www.scitcentral.com

Original Research: Open Access

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

Alexandra Zavaleanu[2], Daniela-Gabriela Glavan[3,†], Andrei Olaru[4], Denissa-Greta Olaru[5], Otilia Margaritescu[6], Aurel Popa-Wagner[1,2*]

[1]*Griffith University School of Medicine, Gold Coast Campus, QLD, USA, 2 Queensland Eye Institute, Brisbane, QLD, Australia*

[2]*Department of Functional Sciences, Center of Clinical and Experimental Medicine, University of Medicine and Pharmacy of Craiova, Romania*

[3]*Psychiatry Clinic Hospital, University of Medicine and Pharmacy of Craiova, Petru Rares Street 2, 200349 Craiova, Romania*

[4]*Department of Ophthalmology, University of Medicine and Pharmacy of Craiova, Romania*

[5] *Medlife Clinic, Department of Ophthalmology, Craiova, Romania*

[6] *Department of Neurosurgery, University of Medicine and Pharmacy of Craiova, Romania*

Received March 16, 2018; Accepted April 4, 2018; Published June 18, 2018

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 exploting 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-

[†]These authors contributed equally to this work.

[*]**Corresponding Author**: Aurel Popa-Wagner**,** Department of Functional Sciences, Center of Clinical and Experimental Medicine, University of Medicine and Pharmacy of Craiova, Romania**;** Email: aurel.popa-wagner@geriatrics-healthyageing.com

Citation: Zavaleanu A, Glavan DG, Olaru A, Olaru D G, Margaritescu O, et al., (2018) Development of New Stem Cell-Seeded Hydrogels to Support the Recovery of Brain Structure and Function after Stroke. J Ageing Restor Med, 1(1): 13-18.

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

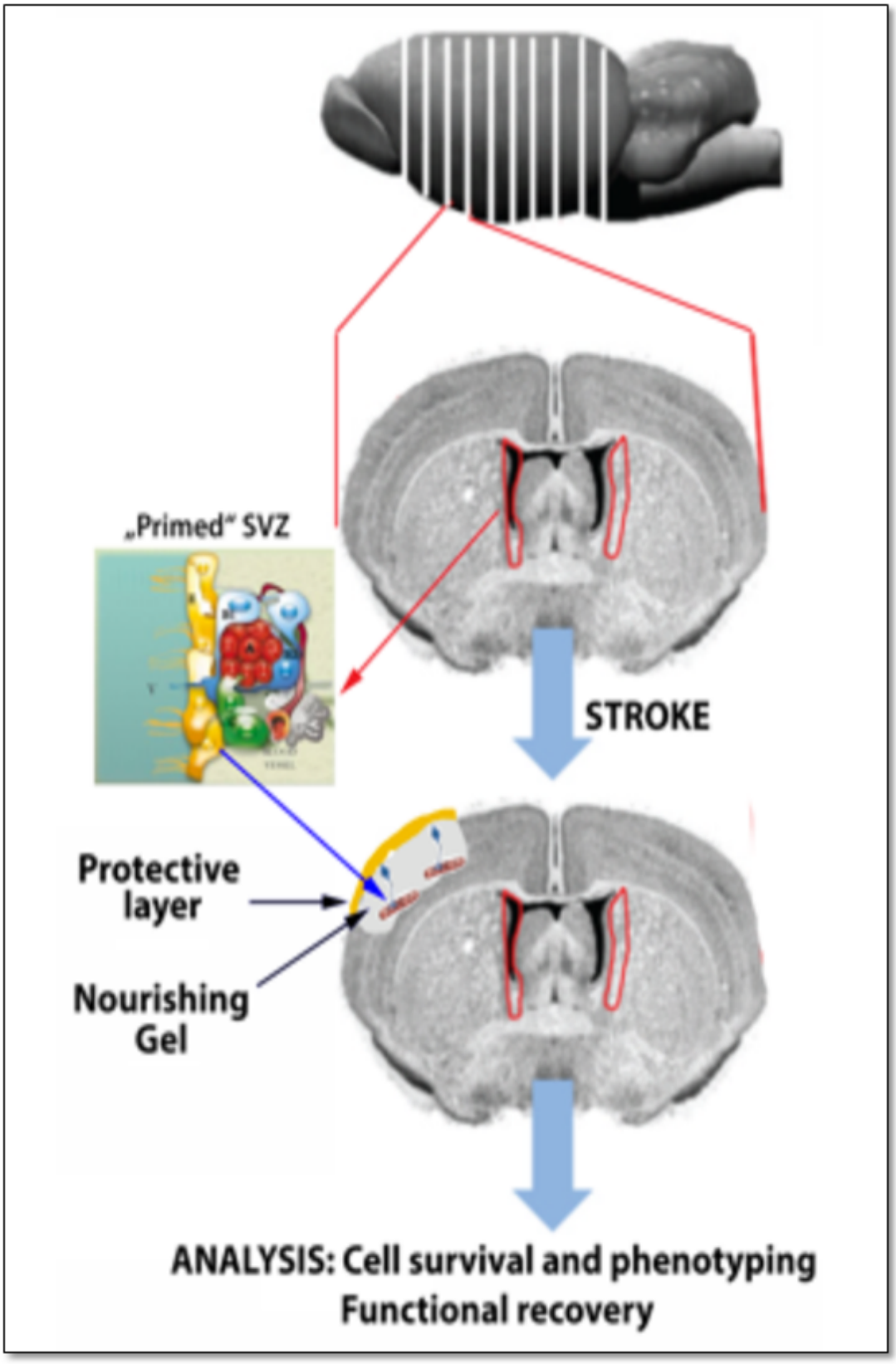

Figure 1. Schematic illustration of the stem cells seeded hydrogels for stroke therapy.

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 re-growth 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 Calcrl, 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 immunhistochemistry 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 strole cavity containing the embedded SVZ will by 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].

REFERENCES

1. Popa-Wagner A, Buga AM and Kokaia Z. Perturbed Cellular Response to Brain Injury During Aging. Aging Research Reviews, 2011; 10(1):71-79. doi: 10.1016/j.arr.2009.10.008.

2. Buga AM, Di Napoli M, Popa-Wagner A. Preclinical models of stroke in aged animals with or without comorbidities: role of neuroinflammation. Biogerontology, 2013; 10(1):71-79. doi: 10.1016/j.arr.2009.10.008.

3. Popa-Wagner A, Schröder E, Walker LC, Kessler C. Beta-Amyloid precursor protein and ss-amyloid peptide immunoreactivity in the rat brain after middle cerebral artery occlusion: effect of age. Stroke, 1998; 29(10):2196-202.

4. Popa-Wagner A, Dinca S., Yalikun L. et al. (2006) Accelerated delimitation of the infarct zone by capillary-derived nestin-positive cells in aged rats. Current Neurovascular Research, 2006; 3: 3-13.

5. Di Napoli M, Godoy DA, Campi V, et al. C-Reactive Protein After Intracerebral Hemorrhage. Time-course, Tissue Localization and Prognosis. Neurology, 2012; 79:660-699.

6. Badan I, Platt D, Kessler C, Popa-Wagner A. Temporal dynamics of degenerative and regenerative events associated with cerebral ischemia in aged rats. Gerontology, 2003; 49(6):356-65. doi:10.1159/000073763

7. Buchhold B, Mogoanta L, Suofu Y, et al. Environmental enrichment improves functional and neuropathological indices following stroke in young and aged rats. Restorative Neurol. Neurosci. 2007; 25: 1–18.

8. Balseanu AT, Buga AM, Catalin B, Wagner DC, Boltze J, Zagrean AM, Reymann K, Schaebitz W, Popa-Wagner A. Multimodal Approaches for Regenerative Stroke Therapies: Combination of Granulocyte Colony-Stimulating Factor with Bone Marrow Mesenchymal Stem Cells is Not Superior to G-CSF Alone. Front Aging Neurosci, 2014; Jun 23;6:130.

9. Buga AM, Margaritescu C, Scholz CJ, et al. Transcriptomics of post-stroke angiogenesis in the aged brain. Front Aging Neurosci., 2014; 6:44. doi:0.3389/fnagi.2014.00044.

10. Buga AM, Scheibe J, Moller K, Ciobanu O, Posel C, Boltze J, Popa-Wagner A. Granulocyte colony-stimulating factor and bone marrow mononuclear cells for stroke treatment in the aged brain. Curr Neurovasc Res, 2015; 12(2):155-62.

11. Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew MV. Astrocyte scar formation aids central nervous system axon regeneration. Nature, 2016; Apr 14;532(7598):195-200. doi: 10.1038/nature17623

12. Popa-Wagner A, Badan I, Walker L, et al. Accelerated infarct development, cytogenesis and apoptosis following transient cerebral ischemia in aged rats. Acta Neuropathol. (Berlin), 2007; 113:277-293.

13. Hermann DM, Chopp M. Promoting brain remodelling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation. Lancet Neurol, 2012; Apr;11(4):369-80. doi: 10.1016/S1474-4422(12)70039-X

14. Honmou O, Onodera R, Sasaki M, Waxman SG, Kocsis JD. Mesenchymal stem cells: therapeutic outlook for stroke. Trends Mol Med, 2012; May;18(5):292-7. doi: 10.1016/j.molmed.2012.02.003

15. Popa-Wagner A, Buga AM, Doeppner TR, Hermann DM. Stem cell therapies in preclinical models of stroke associated with aging. Front Cell Neurosci, 2014; Nov 3;8:347. doi: 10.3389/fncel.2014.00347.

16. Junker H, Suofu Y, Venz S, Sascau M, Herndon JG, Kessler C, Walther R, Popa-Wagner A. Proteomic identification of an upregulated isoform of annexin A3 in the rat brain following reversible cerebral ischemia. Glia, 2007; Dec;55(16):1630-7.

17. Raluca Elena Sandu, Ana-Maria Buga, Adrian Tudor Balseanu, et al. Twenty four hours hypothermia has temporary efficacy in reducing brain infarction and inflammation in aged rats. Neurobiology of Aging, 2016; 38:127-140.

18. Junker H, Suofu Y, Venz S, et al. Proteomic identification of an upregulated isoform of Annexin A3 in the rat brain following reversible cerebral ischemia. Glia, 2007; 55:1630-1637

19. Buga AM, Sascau M, Herndon JG, Kessler K et al. The genomic response of the contralateral cortex to stroke is diminished in the aged rats. J. Cell. Mol. Med., 2008; 12: 2731-2753.

20. Buga AM, C Scholz, S Kumar, et al. Identification of new therapeutic targets by genome-wide analysis of gene expression in the ipsilateral cortex of aged rats after stroke. PLoS ONE, 2012; 7: e50985.

21. Potter W, Kalil RE, Kao WJ. Biomimetic material systems for neural progenitor cell-based therapy. Front Biosci, 2008; Jan 1;13:806-21.

22. Chai P, Napolitano AP, Dean DM, Morgan JR. Dynamics of the self-assembly of complex cellular aggregates on micromolded nonadhesive hydrogels. Tissue Eng, 2007; Aug;13(8):2087-94.

23. Van Wie BJ, Metallo CM, Mohr JC, Detzel CJ, de Pablo JJ, Palecek SP. Engineering the stem cell microenvironment. Biotechnol Prog, 2007; Jan-Feb;23(1):18-23.

24. Nih LR, Moshayedi P, Llorente IL, Berg AR, Cinkornpumin J, Lowry WE, Segura T, Carmichael ST. Engineered HA hydrogel for stem cell transplantation in the brain: Biocompatibility data using a design of experiment approach. Data Brief, 2016; Nov 24;10:202-209.

25. Joseph C, Buga AM, Vintilescu R, Balseanu AT, Moldovan M, Junker H, Walker L, Lotze M, Popa-Wagner A. Prolonged gaseous hypothermia prevents the upregulation of phagocytosis-specific protein Annexin 1 and causes low-amplitude EEG activity in the aged rat brain after cerebral ischemia. J Cereb Blood Flow Metab, 2012; 32:1632-42.

26. Balseanu AT, Buga AM, Catalin B, Wagner DC, Boltze J, Zagrean AM, Reymann K, Schaebitz W, Popa-Wagner A. Multimodal Approaches for Regenerative Stroke Therapies: Combination of Granulocyte Colony-Stimulating Factor with Bone

Marrow Mesenchymal Stem Cells is Not Superior to G-CSF Alone. Front Aging Neurosci, 2014; Jun 23;6:130.

27. Zhong J, Chan A, Morad L, et al. Hydrogel matrix to support stem cell survival after brain transplantation in stroke. Neurorehabil Neural Repair, 2010; 24(7):636-44. doi: 10.1177/1545968310361958.

28. Froget S, Barthelemy E, Guillot F, et al. Wound healing mediator production by human dermal fibroblasts grown within a collagen-GAG matrix for skin repair in humans. Eur Cytokine Netw., 2003; 14(1):60-64.