Brain hydrogel delivery of clustered Vascular Endothelial Growth Factor promotes behavioral recovery after stroke

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Statement of Purpose: Stroke is the leading cause of adult disability in the US. Although tissue regeneration is limited after stroke, angiogenesis and neurogenesis have been described as the main critical phenomena involved in post-stroke tissue regeneration, correlating with improved long-term functional recovery and longer survival of stroke patients. Although the vascular endothelial growth factor (VEGF) appears as the best candidate for therapeutic angiogenesis, its administration after stroke has been limited by poor penetration across the blood-brain barrier, a short half-life time, and severe side effects due to its ability to promote vascular permeability. Recent advances in biopolymer hydrogels have developed gels with extracellular matrix motifs that not only support cell infiltration when injected directly in the infarcted area but also allows a time and space-controlled delivery of growth factors. The Segura laboratory has been focusing for years at developing hyaluronic acid (HA) hydrogels for wound healing purposes and has showed its beneficial effects on tissue regeneration. Recently, we showed that displaying VEGF in a clustered conformation by immobilizing covalently the growth factor on nanoparticles of heparin and controlling the discrete distribution on the particle's surface promotes vascular sprouting. Thus, we hypothesize that different cluster densities of VEGF nanoparticles in HA gel can be used to modulate and promote brain repair and behavioral recovery after stroke via an enhanced angiogenesis in the damaged area.

Methods: Mice were subjected to an cortical ischemic stroke and injected 5 days later with 1) HA-RGD hydrogel alone (Empty gel), 2) HA-RGD plus 200ng of soluble VEGF (Gel + Vs), 3) HA-RGD plus covalently bound VEGF on heparin particles at a low (Gel + lcV) or 4) high (Gel + hcV) clustering density onto heparin nanoparticles surfaces, directly into the stroke cavity. Mice were then submitted to behavioral tests every 4 weeks for 4 months, sacrificed and brain sections were stained for both vascular and axonal networks. In order to identify the axonal and angiogenic mechanism underlying brain tissue repair after gel injection, two additional negative control groups were added: 1) Gel + hcV + Endostatin, a VEGF-independent angiogenesis inhibitor injected in i.p for 10 days after gel injection, and 2) Gel + hcV + CNO, where animals were injected with a recombinant adeno-associated virus vector 5 (AAV5) construct expressing hM4 DREADD receptors (designer receptors exclusively activated by a designer drug), capable of silencing transfected neurons after attachment to the administered drug clozapine-N-oxide (CNO) at 16 weeks, allowing thus to study the direct association between brain activity in the infarcted zone and the behavioral outcome. The 6 animal groups were submitted to 3 different behavioral tests to obtain a quantitative measure of forepaw (Pasta test), forelimb (Cylinder test), and hindlimb (Grid test) motor impairments.

Results: The results showed that the brain transplantation of the hydrogel containing the high cluster of VEGF (hcV) was associated with a significantly enhanced vascular and axonal density in the infarcted are, 4 months after gel implantation. The behavioral assessment showed that the hcV administration enhanced the functional recovery significantly faster than in any other condition tested (up to 8 weeks instead of 16). Interestingly, the effect of hcV on axonal sprouting, vascular growth, and neurological recovery were lost with the administration of endostatin. Similarly, the administration of CNO at 16 weeks was associated with a complete loss of the neurological improvement observed in hcV.

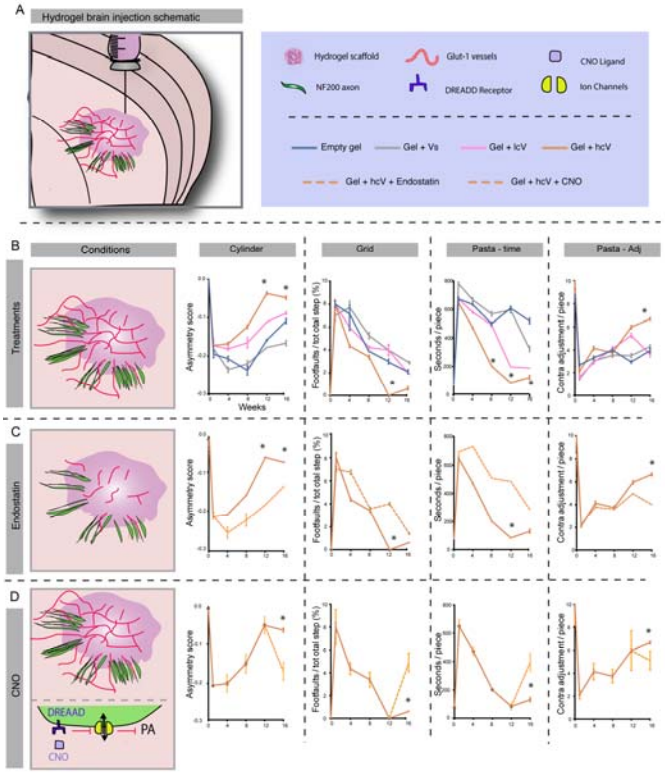

Figure 1: Behavioral assessment for 16 weeks after stroke. (A) Schematic illustration of a coronal brain section and the gel injection into the stroke cavity (A). Behavioral score obtained in the different treatment groups (B), Gel + hcV + Endostatin (C) and Gel + hcV + CNO (D). * indicates $P < 0.05$ vs all other groups (Anova, Tukey's post-hoc test).

Conclusions: This study shows that engineered nanoparticles of clustered VEGF can be used in association with an ECM-derived hydrogel to enhance post-stroke endogenous repair mechanisms such as angiogenesis and axonal sprouting, and promote a faster neurological recovery. This works highlights the importance of interdisciplinary collaborations between engineers, clinicians, and neuroscientist in the development of innovative pro-repair therapies after stroke.