Rebuilding complete vascular network at traumatic brain injury lesion site using in situ crosslinkable hydrogel

Xiaowei Li[1], Xiaoyan Liu[1], Lin Cui [1], Xuejun Wen [1, 2], Ning Zhang [1, 2]
[1]Department of Bioengineering, Clemson University, Clemson, SC, United States
[2]Department of Orthopedic Surgery, Cell Biology, Medical University of South Carolina, Charleston, SC, United States

Introduction: Traumatic brain injury (TBI), an externally inflicted brain trauma that often produces progressive and irreversible damages to the brain and the body beyond the region and extent of the original insult, has remained as one of the most severe and widespread public health problems in the western world in terms of mortality, morbidity, and cost to the society [1]. The treatments to date for TBI have been focused on managing the primary injury, as well as neuroprotection with pharmacological agents to reduce the secondary damage. Many techniques of brain protection such as hypothermia, osmotic therapy, and decompressive craniectomy are employed in concert to achieve the desired ends. Despite the benefit in small numbers of patients, none of these treatments have been translated into clear improvements in the mortality and neurological outcome [2]. This is perhaps due to the inability of these treatments to replace damaged or necrotic nervous tissue and regenerate brain tissue of functional neurons and glial cells. Neurogenesis naturally couples with angiogenesis during endogenous regenerative response to brain injuries [3]. The unique environment required for the association of neurogenesis to angiogenesis is termed "neurovascular niche". In addition to offering metabolic support for neurogenesis and neuronal development, endothelial cells provide humoral support of neuronal production and recruitment in the adult brain regenerative response [4]. Therefore, an important step to regenerate brain tissue at TBI lesion site is to create a well-structured vasculature network that completely fills in the lesion cavity. To this end, several groups have developed strategies of therapeutic angiogenesis, in which endothelial cell mitogens or gene transfer of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), or basic fibroblast growth factor (bFGF) are directly injected into the ischemic region following brain trauma. Despite the ability to accelerate angiogenesis and reduce the ischemic brain injury, administrations of many of these agents have also increased vascular permeability, and thus exacerbated brain edema. Alternatively, we developed a series of functionalized hydrogels based on in-situ gelable, non-immunogenic materials, such as polyethelene glycol (PEG), short laminin peptides and so on. Using these hydrogels, we are able to induce angiogenesis without angiogenic factor delivery and organize 3-D vasculature network that completely fills the TBI lesion site.

Material/Methods: An in situ cross-linkable hydrogel based on multi-Arm PEG, PEG arcylates, modified laminin peptides, and other additives had been developed. Hydrogels formed at physiological conditions due to conjugate addition reactions. Hydrogels with different stiffness were achieved by changing crosslinking density or the ratios of functional groups on multi-arm PEG and PEG acrylate to simulate in vivo mechanical properties. A rheometer (AR1000, TA Instruments Inc.) was used for the rheological characterization of all hydrogel samples. Imunocytochemistry was used to characterize morphology and phenotypic plasticity of mouse neural stem cells (mNSCs) inside the hydrogels. For in vivo studies, unilateral injury of the sensorimotor cortex was produced by controlled cortical impact. Optimized hydrogels was injected into the TBI lesion site 3 days after TBI lesion. Behavior tests were applied to inspect the behavioral recovery after treatments.

Results/Discussion: Figure 1 shows the time sweep profiles of storage modulus (G') for the 10%, 7.5%, 5%, 2.5%, 2%, 1.5%, and 1% hydrogel networks within the small time frame. With increase of PEG concentrations G' is increased accordingly. Higher PEG concentrations also result in faster gelation (Fig.1 insert). In the in vitro cell culture studies, mNSCs can survive and express normal morphology inside hydrogels of 2% PEG (Fig.2A). As to hydrogels with higher PEG concentration, mNSCs can not spread (Fig.2B) and even not survive (Fig.2C) inside the gel. This phenomena result from the fact that much higher PEG concentrations, less pores inside hydrogels, even no pores (data not shown), which result in the nonpermissive conditions for mNSCs. LN peptide conjugated to hydrogels benefits mNSCs growth. With the increase of LN peptide concentrations, more mNSCs can grow in hydrogels. For in vivo study, TBI model is developed through controlled cortical impact on the right side of rat brain. The lesion lies in sensorimotor cortex resulting in impairments in the left forelimb. Both treatment groups have lesions with the similar size at the similar site (Fig.3 A1 and B1). In the control PBS injection group, the lesion site shows cavity. A well-structured vasculature network was rebuilt at the lesion with the injection of hydrogels (Fig.3B3).

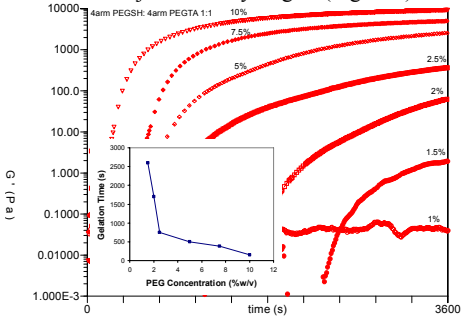

Fig. 1: Rheological characterization of PEG based hydrogels with different concentrations

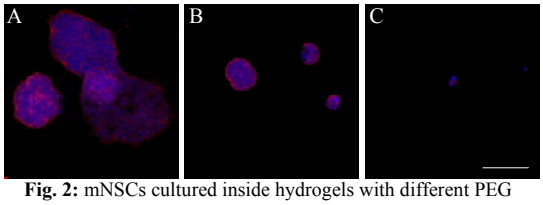

Fig. 2: mNSCs cultured inside hydrogels with different PEG concentrations. A: 2%; B: 5%; C: 7.5%. Red = Actin. Blue = Draq5. Scale bar=100um.

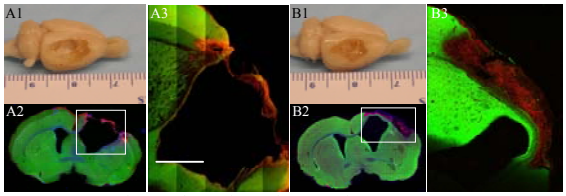

Fig. 3: Macro inspection of specimens of two treatment groups (A: PBS; B: mNSCs with optimized hydrogels). Green = beta III tubulin. Red=Reca-1. Blue = Draq5. Scale bar = 1mm.

Conclusions: Optimized injectable hydrogel system based multi-Arm PEG, PEG arcylates, modified laminin peptides, and other additives can provide permissive environment suitable for the complete reconstruction of vascular network at the TBI lesion site.

References:

1. Goldstein, M., Ann Neurol, 1990. **27**(3): p. 327.
2. McArthur, D.L., et al. Brain Pathol, 2004. **14**(2): p. 185-94.
3. Ohab, J.J., et al., J Neurosci, 2006. **26**(50): p. 13007-16.
4. Leventhal, C., et al., Mol Cell Neurosci, 1999. **13**(6): p. 450-64.