Engineering a Stable Synaptogenic Extracellular Matrix

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Abstract: Synapses are specialized sites of asymmetric cell – cell contact that mediate information transfer between neurons and their targets. Many proteins involved in the recruitment, organization and maintenance of synapses have been identified. Surprisingly, synaptic differentiation does not require a biological membrane surface. Instead, synaptic specializations can form quickly at sites of neurite adhesion to microspheres (beads) coated with synaptogenic proteins or even poly-lysine, a synthetic cationic polypeptide, raising the possibility that functional hemi-synaptic connections could be formed onto designer engineered surfaces. Previous studies examining the stability of synapses formed in brain onto poly-lysine coated beads found they were unstable, degraded, and ultimately replaced by a glial scar. Here, we address the capacity of an extreme biomimetic of poly-lysine, PGB50, a dendritic polyglycerol (dPG)-amine soft matter nanoparticle, to enhance synapse formation in long-term cultures of rat cortical neurons. Microbeads coated with PGB50 exhibit substantially enhanced synaptogenesis and synapse stability compared to poly-lysine. We propose that synaptogenic extracellular matrices could be used to engineer synaptogenic electrodes with enhanced neural-compatibility, reducing glial scaring and inflammation, and allowing for bi-directional communication with neurons through the formation of stable of synaptic specializations.

1 INTRODUCTION

Deficits due to neurodegeneration or injury-induced brain diseases are, ultimately, a direct reflection of the loss of functional synapses. Synapses are specialized sites of asymmetric cell – cell contact that mediate information transfer between neurons and their targets. Many proteins involved in the recruitment, organization, and maintenance of synapses have been identified and the molecular biology of synaptic adhesion is increasingly well understood. Furthermore, neural activity can now be read out to activate muscles or control the movement of robotic limbs (Hochberg et al., 2012; van den Brand et al., 2012). In spite of these advances, contemporary microelectrodes, made of metal or glass, present fundamentally invasive surfaces that neural cells isolate by enclosing in a glial scar.

Synaptic specializations can assemble rapidly following axon – dendrite contact. Surprisingly, the formation of an active pre-synaptic terminal does not require a biological post-synaptic membrane surface. Instead, pre-synaptic specializations can form quickly at sites of axonal adhesion to microspheres (beads) coated with specific lipids or proteins, including the synthetic poly-cationic polypeptide poly-lysine (PLL) (Burry, 1982; Lucido et al., 2009; Gopalakrishnan et al., 2010; Goldman et al., 2013; Suarez et al., 2013).

PLL is a naturally occurring polymer that is susceptible to degradation by several common secreted proteases, including trypsin and cathepsins. Consistent with this, presynaptic specializations formed onto PLL coated beads *in vivo* were not stable but completely degraded within two weeks, replaced by an astrocytic glial scar that isolated the bead (Burry, 1983, 1985). PDL, a protein biomimetic enantiomer of PLL, was developed to resist protease degradation, and thereby enhance its utility as a cell culture substrate. Here, using long-term cultures of embryonic rat cortical neurons we address the capacity of an extreme biomimetic of PLL, PGB50, an ~75 kDa dendritic polyglycerol (dPG)-amine soft matter nanoparticle based on a highly stable and biocompatible polyglycerol scaffold (Hellmund et al., 2015), to enhance synapse formation.

We propose that synaptogenic extracellular matrices may be engineered to enhance biocompati-

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bility and promote the stable formation of synaptic specializations onto manufactured surfaces *in vivo*. Our goal is to engineer synaptogenic electrodes with enhanced neural-compatibility that reduce glial scaring and inflammation, and allow for bi-directional communication with neurons.

2 METHODS

Cell cultures were prepared from cerebral cortex of embryonic day 17–18 (E17–E18). Cells were plated at high density (~40,000 cells/cm^2) and maintained for 14-25 *days in vitro* (DIV) in Neurobasal medium containing 1% B27, 2 mM glutamax and 0.5% N2. Microspheres (7.3 µm polystyrene; Bangs Beads) were washed 3× in PBS (sterile, pH 7.4) before use, then incubated overnight with (50 µg/ml) PLL, PDL and PGB50. Beads were then washed in PBS, pelleted by centrifugation, 7 min at 6500 rpm, and resuspended in culture medium before addition to cultures. Control beads were treated similarly, without coating. Beads were added to cultures at 11 DIV and maintained for an additional 3, 7 or 14 days of incubation (DOI). All images were captured using an Olympus FV1000 confocal microscope. At least 60 Beads were quantified per condition using ImageJ. Corrected total cell fluorescence intensity (CTCF) was calculated for both bead and neurite as: Integrated Density - (Area of selected cell X Mean fluorescence of background), and fold changes in CTCF of bead/neurite plotted. Data are mean ± SEM, Two-way ANOVA followed by a *post hoc* test was used to calculate *p* values.

3 RESULTS

3.1 PGB50 Enhances Synapse Formation

Using long-term cultures of embryonic rat cortical neurons, we tested and compared the efficacy of beads coated with PLL, PDL or PGB50 to induce synapse formation and promote synapse stability.
Immunoreactive CTCF for synaptophysin and PSD-95, marking pre- and post-synaptic specializations, was significantly enhanced for PGB50-coated beads when compared to Control, PLL- and PDL-coated beads (Figures 1A and 1B). These results reveal more effective induction of long-term stable synaptic specializations by PGB-50 compared to either PLL or PDL.

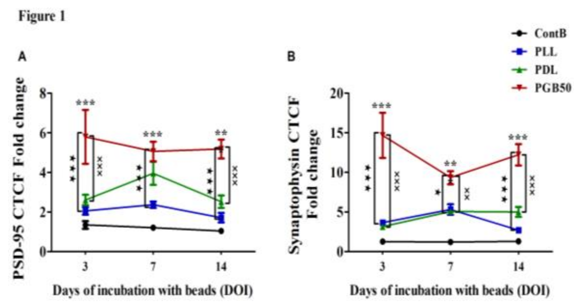

Figure 1: PGB50 Enhances Synapse Formation. Quantification of corrected total cell fluorescence intensity (CTCF) of A. The post-synaptic marker; PSD-95 and B. The pre-synaptic marker; Synaptophysin. Neuronal cultures were incubated with control, PLL-, PDL- or PGB50-coated beads for 3, 7 and 14 days of incubation (DOI). ***$p < 0.001$ vs. control, ★ $p < 0.05$, ★★★ $p < 0.001$ vs. PLL, xx $p < 0.01$, xxx $p < 0.001$ vs. PDL.

3.2 PGB50 Enhances Synaptic Specialization

The formation of synaptic specializations, with a post-synaptic bouton localized adjacent to its pre-synaptic counterpart, is essential for synapse formation, maturation and function. To test whether PGB50, PLL and PDL promote local synapse formation, we quantified the overlap of presynaptic synaptophysin with postsynaptic PSD-95, within a 3D volume around the beads (voxels).

The overlap of PSD-95 and synaptophysin was enhanced by all coatings compared to control beads (Figure 2). PGB50-coated beads exhibited signify-cantly higher numbers of voxels positive for both

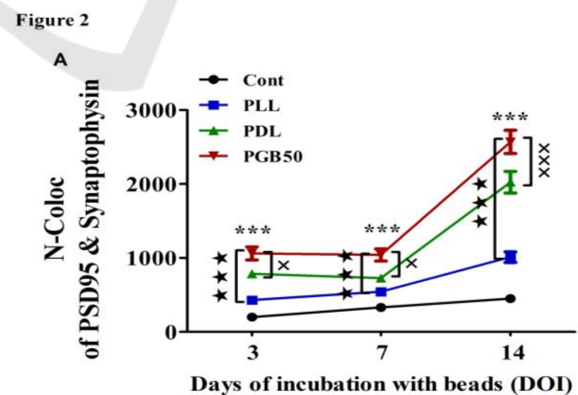

Figure 2: PGB50 Enhances Synaptic Specialization. Quantification of number of co-labeled voxels (N-Coloc) of the post-synaptic marker; PSD-95 and the pre-synaptic marker; Synaptophysin. Neuronal cultures were incubated with control, PLL-, PDL- or PGB50- coated beads for 3, 7 and 14 days of incubation (DOI). ***$p < 0.001$ vs. control, ★★★ $p < 0.001$ vs. PLL, x $p < 0.05$, xxx $p < 0.001$ vs. PDL.

PSD-95 and synaptophysin at all time points examined. These results suggest a greater capacity of PGB50 compared to PLL and PDL to initiate and support the local formation of synapses.

4 DISCUSSION

Studies carried out in the 1980s, addressing the function of synaptogenic poly-cationic polymers, found that simple beads coated with PLL had the capacity to direct the formation of presynaptic specializations *in vitro* and *in vivo*, but the synapses formed did not persist, and within a few days *in vivo* were displaced by an astrocytic scar (Burry, 1983, 1985). Although these findings supported the idea that non-neuronal surfaces, when decorated with the "correct" chemical signals could induce the formation of synaptic specializations, the short lifetime of the synapses formed was fundamentally problematic for translational applications. Here, we provide evidence for enhanced synapse formation and stability induced by the dendritic polyglycerol PGB50, a highly stable non-protein molecular biomimic of poly-lysine.

Our ongoing studies aim to enhance the function and stability of synapses formed onto modified synaptogenic surfaces *in vivo,* and develop approaches to stimulate and record from these surfaces. We aim to promote the formation of adhesive contacts by axons and dendrites that will be inherently more stable and better positioned to record neuronal activity than is currently possible using conventional electrodes. Our findings suggest that the hemi-synaptic specializations formed onto the dendritic polyglycerol surface will in turn induce synapse formation by adjacent axons and dendrites, resulting in the development of a dense local web of synaptic connections surrounding the electrode. Ultimately such an implant would achieve functional integration into the neuronal network. We envision that such implanted synaptogenic interfaces would be broadly applicable to extend the function of the injured or diseased human nervous system.

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