# Enhanced Nerve Cell Proliferation and Differentiation Using Hydrogels Grafted with Photo-polymerizable Poly-L-Lysine

Lei Cai and Shanfeng Wang

Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN 37996

**Statement of Purpose:** Injectable and photo-crosslinkable hydrogels, mimicking properties of extracellular matrices (ECM) in brain and nerve tissues, have been widely used to assist regeneration of nervous systems[1]. Three photo-crosslinkable poly(ethylene glycol) diacrylates (PEGDAs) with different molecular weights have been synthesized by us using $K_2CO_3$ as the proton scavenger[2,3]. Then PEGDAs were modified by crosslinking with polymerizable poly-L-Lysine (PLL), which had one double bond end group from the initiator allylamine in its synthesis. By varying the molecular weight that determined the crosslinking density, PEGDA-based hydrogels exhibited controllable mechanical properties, which could be used to regulate attachment, proliferation, and differentiation of rat pheochromocytoma (PC12) cells and neural stem cells (NSCs).

**Methods:** Three PEGDAs were synthesized using polyethylene glycol (PEG) precursors with nominal $M_n$ of 1k, 3k, and 10k g.mol$^{-1}$ and acryloyl chloride in the presence of $K_2CO_3$. The number-average and weight-average molecular weights of these PEGDAs are 1450, 5450, 14500 g.mol$^{-1}$ and 1560, 5640, 15300 g.mol$^{-1}$, respectively. Photo-polymerizable PLL ($M_n$ = 3060 g.mol$^{-1}$, $M_w$ = 3750 g.mol$^{-1}$) was synthesized via ring opening polymerization of Z-L-Lys-*N*-carboxyanhydride using allylamine as the initiator (Figure 1)[4]. In this study, 1 wt% PLL in PEGDA was used to form a 30 wt% aqueous precursor solution and was then photo-crosslinked.

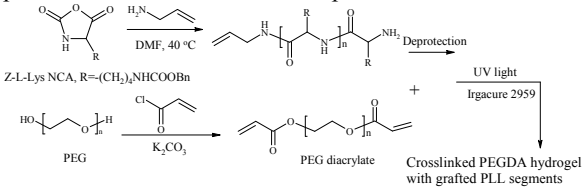


**Figure 1.** Synthesis and crosslinking of PLL and PEGDA

**Results:** The synthetic route of PEGDAs using $K_2CO_3$ as the proton scavenger could significantly simplify purification steps and avoid complex formation between acryloyl chloride and triethylamine, a widely used proton scavenger[3]. All photo-crosslinked PEGDA hydrogels showed characteristic curves as polymer networks with frequency-independent storage modulus $G'$, which was always greater than loss modulus $G''$. $G'$ increased from 10 kPa for PEGDA10k to 80 kPa for PEGDA3k and 250 kPa for PEGDA1k, showing efficient controllability using the crosslinking density. PC12 cells consistently favored softer substrates by demonstrating higher attachment, faster proliferation, and more neurite extension in the presence of nerve growth factor (Figure 2b,e). Tethered PLL chains with positive charges dramatically enhanced PC12 cell attachment, proliferation, and neurite extension. The same trend on mechanical stimulus was found in NSC proliferation by showing larger neurospheres on softer or PLL-grafted hydrogels (Figure 2c,e). Further, substrate stiffness was found to affect preference of the differentiated forms of NSCs by showing more neurons and fewer astrocytes on softer hydrogels. Grafted PLL chains provided more binding ligands that could significantly improve percentage of neurons and glial-natured astrocytes differentiated from NSCs (Figure 2d,e).

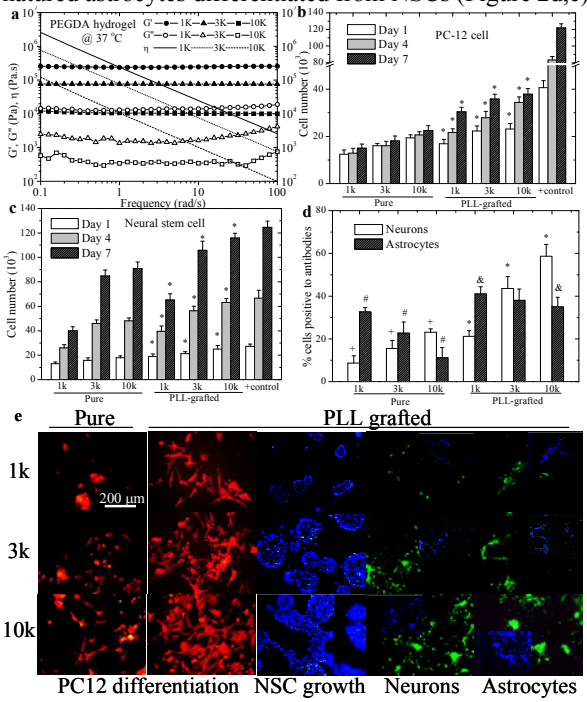


**Figure 2.** (a) $G'$, $G''$, and viscosity $\eta$ vs. frequency for crosslinked PEGDA-based hydrogels at 37 °C. (b) PC12 and (c) NSC proliferation at days 1, 4, and 7, and (d) NSC differentiation on pristine and PLL-grafted PEGDA hydrogels.(e) Images of neurites (red) for PC12 differentiation, nuclei (blue) for NSC growth, and neurons or astrocytes (green) for NSC differentiation (Inset: nuclei). Scale bar of 200 μm is applicable to all in (e). *, $^{\&}$: $p < 0.05$ between each other and compared to the corresponding data on pristine PEGDA hydrogels. $^{+}$, $^{\#}$: $p < 0.05$ between two marked samples.

**Conclusions:** Three PEGDAs with different molecular weights have been synthesized in the presence of $K_2CO_3$ for achieving hydrogels with distinct stiffnesses. Softer or PLL tethered PEGDA hydrogels could greatly promote PC12 and NSC proliferation and differentiation. Both mechanical and chemical factors are crucial in achieving suitable microenvironment to support nerve cell functions. PEGDA hydrogels grafted with newly synthesized photo-polymerizable PLL in this study are promising injectable materials for nerve repair and regeneration.

**References:**

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4. Motala-Timol, S. *Macromolecules* **2008**, *41*, 5571.