A dopamine–based poly(aminoglycerol ester) induces significant neurite extension in differentiated PC12 cells and rat DRGs

Jin Gao[1]*, Yu Mi Kim[1, 2]*, Herna Coe[1], Blaine Zern[1], Barbara Sheppard[3] and Yadong Wang[1]
[1]Department of Biomedical Engineering and Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA. [2]Department of Biological and Medical Engineering, Kyungpook National University, Daegu, Korea. [3]College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA.
*J.G. and Y.K. contributed equally to this work.

Statement of Purpose: Biomaterials are widely used in modern medicine. Recently, significant advances have been made to impart biological activity to biomaterials. Most of the existing bioactive materials are derived from extracellular matrix or are modified with extracellular matrix motifs. The most widely used extra-cellular matrix motifs such as Arg-Gly–Asp. In addition to cell-extracellular matrix interactions, cell differentiation and survival also depend on constant interactions with other cells through a plethora of messenger molecules. The biomaterial reported here is designed to use dopamine, one of the neurotransmitters in the nervous system, to impart bioactivities to resultant biodegradable polymers.

Methods: The dopamine based polymer (PCD) was synthesized with equimolar amount of diglycidyl 1,2-cyclohexanedicarboxylate and dopamine in DMF with 0.1% $Mg(ClO_4)_2$ under N_2 with constant stirring, after heating at 90 °C for 7 days, then precipitated in diethyl ether. The precipitate was subjected to a quick washed with deionized, then lyophilized. The polymer was then characterized by NMR spectra, FTIR spectra, differential scanning calorimetry, and mass spectrometry. The material's capability at promoting neurite outgrowth *in vitro* was evaluated using explanted rat dorsal root ganglions (DRGs) and rat pheochromocytoma (PC12) cells that have been widely used in studying neuronal communication and interaction between biomaterials and neurons. Biocompatibility of the polymer with nervous tissue *ex vivo* and *in vivo* was performed by culturing rat dorsal root ganglion neuron on polymer surface, and by implanting immediately adjacent to rat sciatic nerves, respectively.

Results/Discussion: PCD was a pale yellow powder soluble in N,N-dimethylforamide, low molecular weight alcohols and ketones, but not water. The half-life of PCD in phosphate buffered saline solution (PBS) was approximately 50 days at 37 °C. Cell proliferation on PCD resembled that of Poly (D-lysine) (PDL) and was significantly better than that of tissue culture polystyrene surface (TCPS) with dopamine added to the culture medium. The introduction of free dopamine appeared to inhibit the proliferation of PC12 cells, which agrees with previous reports. The number of PC12 cells exhibiting typical neuronal morphology was significantly higher on PCD than on PCY (a control polymer based on tyrosine), PDL, and laminin surfaces (**Fig.1**). A preliminary study using rat DRG explants revealed that they adhered well and displayed significant neurite outgrowth on PCD. A large number of long, loosely-bundled neurites sprouted out from the DRGs on both PCD and PDL surfaces. The neurites on PCD exhibited an "arborizing" mode of growth with extensive branching, whereas the neurites on PDL appeared more linear. A preliminary *in vivo* biocompatibility study indicated that PCD pellets did not cause nerve degeneration or fibrous encapsulation when implanted immediately adjacent to rat sciatic nerves.

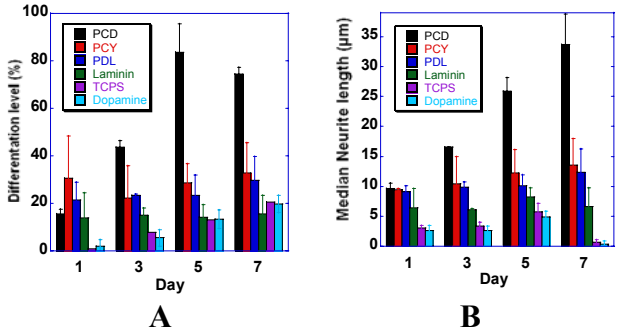

Figure 1. NGF primed PC12 cells grew longer neurites and exhibited higher differentiation rate on PCD versus PCY, PDL, laminin, TCPS and TCPS with free dopamine. (A) Cells differentiation; (B) The median neurite length.

Conclusions: We have designed and synthesized a bioactive and biodegradable biomaterial that derived its biological activity from dopamine. NGF-primed PC12 cells and explanted rat DRGs attached well and displayed substantial neurite outgrowth on the polymer surface. Further, PCD promoted more vigorous neurite outgrowth in PC12 cells than tissue culture polystyrene, laminin and PDL. PCD did not cause nerve degeneration or fibrous encapsulation when implanted immediately adjacent to the rat sciatic nerves. This work is a first step towards creating a diverse family of bioactive materials using small chemical messengers as monomers.

References: Snyder SH. Cell. 2006;125:13-15.
Grigoriadis N. *et. al.* Dev Brain Res. 2004;153:79-87.
Van Kesteren RE *et. al.*Rev Neurosci. 2003;14: 217-231.
Koshimura K *et. al.* J Neurosci Res. 2000; 62:112-119.
Angelastro JM *et. al.* Proc Natl Acad Sci USA. 2000; 97:10424-10429.