Long term delivery of nerve growth factor by nano/microparticles for enhancing peripheral nerve regeneration

Anup D. Sharma[1,4], Metin Uz[1], Donald S. Sakaguchi[2,3,4], and Surya K. Mallapragada[1,4].
[1]Department of Chemical and Biological Engineering, [2]Department of Genetics, Development, and Cell Biology, [3]Biology Program, [4]Neuroscience Program, Iowa State University, Ames, Iowa

Statement of Purpose: Delivery of neurotrophic factors to growing axons is helpful in enhancing nerve regeneration. There are various ways of delivering neurotrophic factors such as direct release from nerve regeneration conduits, transplantation of cells hyper secreting neurotrophic factors, and micro/nano delivery devices[1]. Nerve growth factor (NGF) is the most commonly used neurotrophic factor for sciatic nerve regeneration. In the current study, we are focusing on using biocompatible and biodegradable polyanhydrides polymers [2-4] for fabrication of NGFβ encapsulated nano/microparticles. Polyanhydrides nanoparticles have been successfully used for encapsulating various proteins and also for providing a sustained release of protein during the time of biodegradation. There are certain advantages of using polyanhydrides for making these protein-secreting nano/microparticles as they degrade by mechanisms of surface erosion unlike polyesters (poly lactic acid) which undergoes bulk degradation by acidic hydrolysis. Also the degradation products of polyanhydrides cause less acidity in the microenvironment as compared to the polyesters. Here we are planning to use copolymers of 1, 6-bis (p-carboxyphenoxy) hexane (CPH), 1, 8-bis (p-carboxyphenoxy)-3, 6-dioxaoctane (CPTEG) and sebacic acid (SA) to fabricate micro/nanoparticles for controlled release of NGF.

Methods: CPH: SA and CPTEG: CPH copolymers were synthesized from the corresponding monomers via a melt polycondensation reaction. Nuclear magnetic resonance spectroscopy (NMR) was used for resolving the structure and determining the molecular weight of the copolymer. CPH: SA and CPTEG: CPH nanoparticles were fabricated by Solid/Oil/Oil (S/O/O) emulsion method where copolymers were dissolved in methylene chloride first and then rapidly nanoprecipitated in anti-solvent (pentane) to obtain the nanoparticles. In a similar fashion, NGFβ and polymer was dissolved in methylene chloride and precipitated in anti-solvent to obtain NGFβ encapsulated nanoparticles. Amount of NGFβ used for encapsulation was 0.1% the weight of the polymer used for encapsulation. Spray drying was also used for fabricating CPH: SA and CPTEG:CPH microparticles to increase the encapsulation efficiency. Scanning electron microscopy (SEM) imaging was performed to get an idea of the dimensions and structure of nanoparticles obtained. Figure 1 shows SEM images of 20:80 CPH: SA nanoparticles with and without encapsulated NGFβ.

Results: No release was observed from S/O/O nano and microparticles of 20:80 CPH: SA and 20:80 CPTEG: CPH. A small release was seen from spray dried microparticles of 20:80 CPH: SA and 20:80 CPTEG: CPH. 50:50 CPTEG: CPH particles looked most promising and released the NGFβ for upto a month. To assess the cytotoxicity of these micro/nanoparticles, GFP expressing – Mesenchymal Stem Cells were incubated for 48 hours with particles before performing the CCK8 cell viability assay. No to very little cytotoxicity was observed for 50:50 CPTEG: CPH particles at a concentration of 200μg/ml or less. Also, PC12 cells neurite extension assay showed that NGFβ released from 50:50 CPTEG:CPH microparticles was indeed bioactive and showed a neurite extension pattern similar to that of using recombinant NGF in PC12 maintenance media. PC12 cells grown with blank microparticles and PC12 cells growing in maintenance media alone had a very little-to-no neurite extension.

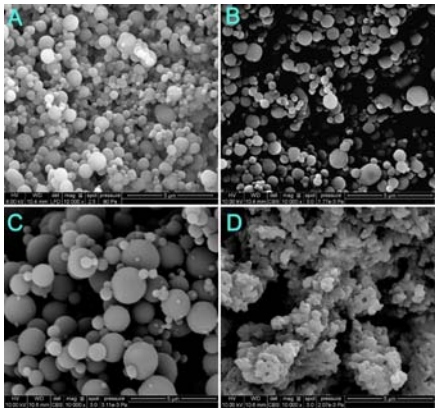

Figure 1. SEM images of various types of nano/microparticles. (A) 20:80 CPH: SA nanoparticles without NGF fabricated by Solid/Oil/Oil method (B) 20:80 CPH: SA nanoparticles with 0.1% NGFβ fabricated by Solid/Oil/Oil method. (C) 20:80 CPH: SA microparticles with 0.05% NGFβ fabricated by Spray drying. (D) 50:50 CPTEG: CPH microparticles with 0.1% NGFβ fabricated by Solid/Oil/Oil method.

Conclusions: This work can lead to fabrication of potentially effective NGFβ releasing biodegradable polyanhydrides micro/nanoparticles which can facilitate and enhance peripheral nerve regeneration.

References:

1. Babensee, J.E., L.V. McIntire, and A.G. Mikos, *Growth factor delivery for tissue engineering*. Pharm Res, 2000. 17(5): p. 497-504.
2. Torres, M.P., et al., *Synthesis and characterization of novel polyanhydrides with tailored erosion mechanisms*. J Biomed Mater Res A, 2006. 76(1): p. 102-10.
3. Determan, A.S., et al., *Encapsulation, stabilization, and release of BSA-FITC from polyanhydride microspheres*. J Control Release, 2004. 100(1): p. 97-109.
4. Torres, M.P., et al., *Amphiphilic polyanhydrides for protein stabilization and release*. Biomaterials, 2007. 28(1): p. 108-16.