Development of 3D Printed hydrogel scaffold with core-shell nanoparticles for nerve regeneration

Se-Jun Lee[1], Wei Zhu[1] and Lijie Grace Zhang[1,2]
[1]Department of Mechanical and Aerospace Engineering, The George Washington University
[2]Department of Medicine, The George Washington University

Statement of Purpose: Neural tissue engineering is being explored in an effort to develop artificial nerve scaffolds to overcome the limitation of autologous transplantation. Particularly, advancement in both 3D scaffold fabrication strategies and nanotechnology has inspired this field into a new era. In this study, we developed a novel nerve guidance scaffold by integrating novel stereolithography (SL) based 3D printing and bioactive nanomaterials. In particular, poly lactic-co-glycolic acid (PLGA) core-shell nanoparticles with encapsulated bovine serum albumin (BSA) were fabricated via a coaxial electrospraying technique. The method enables the separation of organic and aqueous phases and thus incorporation of biologically active components into the aqueous phase without exposing them to harmful organic solvents. Then, the nanoparticles were embedded inside biocompatible poly (ethylene glycol) diacrylate (PEG-DA) hydrogel solution and printed into a biomimetic nerve guidance scaffold by a SL based 3D bioprinter.

Methods: Core-shell nanoparticles were fabricated by coaxial electrospraying method in assist of a core-shell needle. Core solution comprised of 1.0% (w/v) BSA in distilled water. PLGA was dissolved in acetone in a concentration of 2.5% (w/v) as shell solution. Two solutions were fed at a flow rate of 2.5mL/h through individual syringe. A 9 ~ 15 kV voltage was applied to obtain stable cone jet mode of nanoparticles.

Scaffolds were designed as square pattern with small, medium, and large and small pores geometry (corresponding to 44%, 56%, and 68% porosity) using computer aided design software. For the nanoparticles embedded scaffolds fabrication, lyophilized nanoparticles were blended with printable solution by ultrasonication in concentrations of 0.1%, 0.5%, and 1%, respectively. The prepared solutions were crosslinked by ~110 mW UV laser radiation with a printing speed of 25 mm/s. All prepared scaffolds were sterilized by ethanol followed by UV light and stored in PBS for further cell assay.

PC-12 cell line (ATCC) was utilized to evaluate the cell response on printed nerve scaffolds. The 4 h cell attachment and 2, 4, and 6 days proliferation were studied. All scaffolds were coated with poly-L-lysine overnight in order to enhance cellular attachment. PC-12 cells were seeded on prewetted scaffolds at a density of 5 × 10^4 cells per scaffold. Cell viability was quantified by Alamar Blue assay.

Results: **Figure 1** shows the morphologies of nanoparticles and printed scaffolds. 4 h cell adhesion study showed the scaffolds with 68% porosity can significantly improve cell attachment compared to other two groups (**Figure 2**). Then three more groups of scaffolds (68% porosity) with different concentration of BSA/PLGA nanoparticles were prepared for proliferation study. Compared to control group, PC-12 cells proliferate significantly on scaffolds with nanoparticles (**Figure 3**).

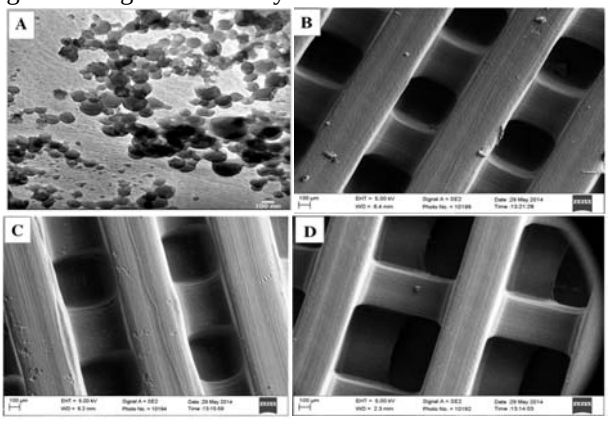

Figure 1. (A) Transmission electron microscopy image of nanoparticles, and scanning electron microscopy images of scaffolds with 44% (B), 56% (C) and 68% porosity (D).

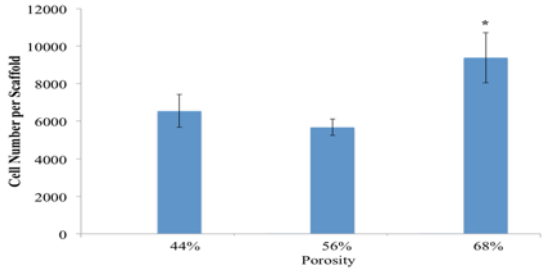

Figure 2. PC-12 cells adhesion on printed scaffolds with various porosity after 4 h of culture. Data are mean ± standard error of the mean; n=9. *p<0.05 when compared to all other scaffolds.

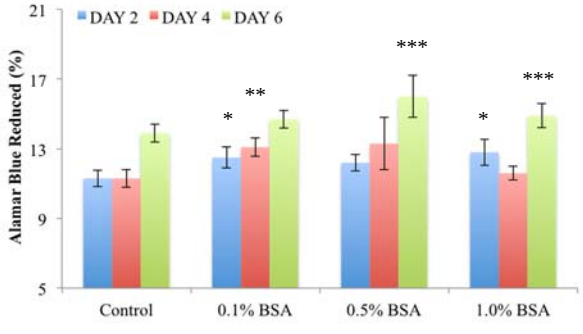

Figure 3. Enhanced PC-12 cell proliferation in 3D printed scaffolds with BSA/PLGA nanoparticles after 6 days culture. Data are mean ± standard error of the mean, n=6; *p<0.05 when compared to control group (without nanoparticles) at day 2, **p<0.05 when compared to control group at day 4, ***p<0.05 when compared to control group at day 6.

Conclusions: A series of nerve scaffolds with controlled porous structure were fabricated using 3D printing method. Meanwhile, bioactive factor nano delivery system was effectively incorporated inside scaffolds and improved neural cell adhesion and proliferation, thus promising for improved neural regeneration.