In-vivo Biocompatibility and Toxicity of Single Walled Carbon Nanotube Composites for Bone Tissue Engineering

Ashim Gupta[1], Benjamin J. Main[1,2], Teresa A. Liberati[1], Melissa H. Roberts[1], Anish G.R. Potty[1], Saadiq F. El-Amin III[1]
[1]Southern Illinois University, School of Medicine, Springfield, IL, USA; [2]University of Illinois at Springfield, Springfield, IL, USA.

Statement of Purpose: Bone defects and non-unions caused by trauma, tumor-resection, pathological deformation and peri-prosthetic fractures pose a great challenge in the field of orthopaedics. Traditionally, these injuries have been treated using autografts and allografts, both of which have restrictions. Bone Tissue Engineering (BTE) has evolved as a means to develop a viable bone graft alternative. In our previous study[1], we demonstrated that reinforcing poly lactic-co-glycolic acid (PLAGA) with Single Walled Carbon Nanotubes (SWCNT), producing a novel SWCNT/PLAGA composite, imparted beneficial cell growth and gene expression. The study[1] also demonstrated that the addition of 10mg SWCNT resulted in the highest rate of cell proliferation. Further, our unpublished study demonstrated that a 3-D SWCNT/PLAGA composite with 10mg SWCNT displayed greater cell proliferation and mechanical strength than that of the PLAGA control. For use in clinical applications, these composites must be biocompatible and non-toxic. The goal of this study was to evaluate the in-vivo biocompatibility and toxicity of SWCNT/PLAGA composites via S.C. implant in a rat model. We hypothesized that SWCNT/PLAGA composites are biocompatible and non-toxic, and are ideal candidates for BTE.

Methods: SWCNT/PLAGA (using 10mg SWCNT) and PLAGA only composites were fabricated using solvent evaporation technique as described in our previous study[1]. 6-week old SD rats were implanted subcutaneously with 12mm diameter SWCNT/PLAGA and PLAGA composites (two disks each, 15mm apart) with the sham surgery group serving as a control (5animals/group/time point). Rats were humanely euthanized at 2, 4, 8 and 12 weeks post-implantation. All of the animals were observed for morbidity and overt toxicity. Individual body weights and food consumption were measured on day 1, 3 and 7 post-surgery for the first week and weekly thereafter. Urinalysis was performed from 5animals/group once at 12 weeks post-implantation. The parameters such as pH, specific gravity, leukocyte, nitrite, protein, ketone etc. were determined using an Urispec^{TM} 11-way kit. At euthanasia at 2, 4, 8 and 12 weeks, whole blood was collected from the heart and hematology was performed on an automated hematology machine examining WBC count, RBC count, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin and platelet count. WBC differential counts including lymphocyte, neutrophil, eosinophil, basophil and monocyte were determined from blood smears stained with Wright-Giemsa. Major organs (i.e. heart, lungs, liver, spleen, kidneys and adrenal glands) were collected, weighed and observed for macroscopic abnormalities. All the organs were fixed and sent to the pathologist for further analysis and histology. In addition, the tissue surrounding the implants, along with the implants, were collected and graded for degree of inflammation. Statistical analysis using ANOVA was performed. The results were considered significant if $p < 0.05$. All animal use was conducted under an approved protocol reviewed by the animal care and use committee at SIU-SOM.

Results: SWCNT/PLAGA and PLAGA composites were successfully fabricated and implanted in the SD rats. No composite-related toxicity or biocompatibility issues were observed in morbidity, clinical signs, body weight, food consumption, urinalysis and hematology by 12 weeks. All rats gained weight appropriately. No macroscopic abnormalities were noted in any of the animals by 12 weeks. S.C. tissue surrounding the implants appeared grossly normal with no overt evidence of inflammation and all incision sites were healed (Figure 1). Further analysis is currently being conducted.

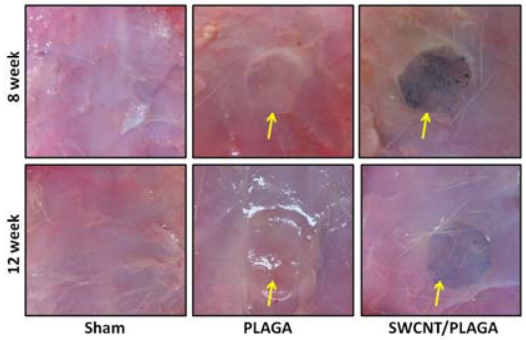

Figure1. Post-necroscopy images of S.C. tissue surrounding the implants (Sham, PLAGA and SWCNT/PLAGA) at 8 and 12 weeks.

Conclusions: Biodegradable composites are of interest in clinical medicine because of their biocompatibility, bioresorbability, non-immunogenicity, and high mechanical strength, essentially circumventing the need to surgically remove the implanted scaffold. SWCNT/PLAGA composites are ideal for BTE. We demonstrated in our previous published[1] and unpublished studies that incorporation of SWCNT to PLAGA significantly enhances cell proliferation and mechanical strength. The results obtained from this study will demonstrate the potential of SWCNT/PLAGA composites for musculoskeletal regeneration and will offer a novel substitute for BTE. Future studies will be designed to evaluate the efficacy of SWCNT/PLAGA composites in bone regeneration in a rabbit model. As interest in carbon nanotube technology increases, studies must be performed to fully evaluate these novel materials at a nonclinical level to assess their safety. The ability to produce composites capable of promoting bone growth will have a significant impact on tissue regeneration and will allow greater functional recovery in injured patients.

References: 1. Gupta A, Woods MD, Illingworth KD, Niemeier R, Schafer I, Cady C, Filip P, El-Amin SF 3rd. Single walled carbon nanotube composites for bone tissue engineering. J Orthop Res 2013; 31(9):1374-81.