In-vitro Evaluation of Three Dimensional Single Walled Carbon Nanotube Composites for Bone Tissue Engineering

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Statement of Purpose: Bone related injuries are among the most common orthopaedic injuries and account for more than 3 million surgeries annually. More than half of these surgeries require bone grafting by either autograft or allograft. To alleviate the limitations posed by autografts and allografts, bone tissue engineering (BTE) has evolved as an alternative strategy to develop bone grafts. In our previous study[1] we demonstrated that addition of Single Walled Carbon Nanotubes (SWCNT) to Poly lactic-co-glycolic acid (PLAGA) formed a SWCNT/PLAGA composite with beneficial cellular growth capabilities and gene expression; and that the addition of 10mg SWCNT resulted in the highest rate of cell proliferation. The purpose of this study was to develop 3-D SWCNT/PLAGA composites using 10mg SWCNT, to determine the mechanical strength of the composites and to evaluate the biocompatibility of MC3T3-E1 cells. We hypothesized that the 3-D SWCNT/PLAGA composites can be designed and optimized to support MC3T3-E1 cell growth, possess adequate mechanical strength, and can be applied for use in BTE.

Methods: SWCNT/PLAGA (using 10mg SWCNT) and PLAGA microspheres were prepared by an oil-in-water emulsion method. SWCNT/PLAGA and PLAGA composites were fabricated via a thermal sintering technique. Scanning Electron Microscopy (SEM) was performed to study the surface and to determine the diameter of the SWCNT/PLAGA and PLAGA microspheres, and to characterize the fabricated composites. Mechanical testing of the composites was carried out using an Instron 5869 at 10% strain/min under physiological conditions to compute the compressive modulus and ultimate compressive strength. Cell adhesion and morphology studies using Immunofluorescence staining and SEM, and cell survival using a Live/Dead assay kit were performed to evaluate biocompatibility. Cell Proliferation studies using a CyQUANT® cell proliferation assay and gene expression analysis using real time PCR were performed. Mean $\pm$ SEM (Standard error of mean) values along with statistical analysis using ANOVA were performed. Mechanical Testing was done with n=6 and statistical differences were determined using a two-tailed, unpaired Student's t-test where two groups were compared. A one-way analysis of variance (ANOVA) with Turkey's test post hoc was performed. The results were considered significant when $p < 0.05$.

Results: The SEM images for PLAGA and SWCNT/PLAGA microspheres demonstrated uniform shape and smooth surface with size ranging from 250-750µm. The SEM micrographs for PLAGA and SWCNT/PLAGA composites showed bonding of the microspheres in a random packing manner with their spacing maintained. SWCNT/PLAGA composites yielded a significantly greater compressive modulus (3-fold) and ultimate compressive strength (4-fold) compared to PLAGA composites (Figure1A and 1B). Immunofluorescence staining and SEM revealed that MC3T3-E1 cells adhered to and grew upon both of the surfaces, and exhibited normal, non-stressed morphology (flat and polygonal). Live/Dead assay demonstrated that cells survived on these composites over a period of 21 days. Higher cell proliferation rate was observed on SWCNT/PLAGA compared to PLAGA and the negative control BGS at all time intervals (day 7, 14 and 21). Gene expression analysis is currently being conducted.

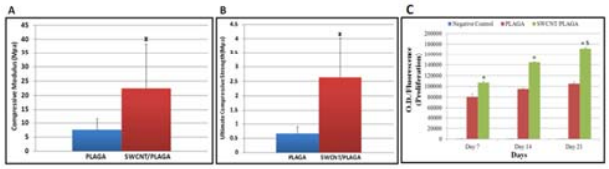

Figure1. (A) Compressive Modulus and (B) Ultimate Compressive Strength of PLAGA and SWCNT/PLAGA composites. * represents significant difference ($p<0.05$) compared to PLAGA. (C) Cell proliferation assay for proliferation of MC3T3-E1cells cultured on PLAGA and SWCNT/PLAGA composites. Data represents Mean $\pm$ SEM and * represents significant difference in proliferation on SWCNT/PLAGA composites compared to PLAGA composites at the same time point at significance level $p< 0.05$. $ is significant over all groups.

Conclusions: Our results demonstrated that SWCNT/PLAGA composites promoted cell adhesion, growth, and survival, and have a higher mechanical strength and cell proliferation rate than PLAGA composites. The findings from this study demonstrated the potential of SWCNT/PLAGA composites for BTE and musculoskeletal regeneration. In order to for this novel SWCNT/PLAGA to serve as a functional bone substitute, future studies must evaluate the porosity of these composites as well as in-vivo biocompatibility and toxicity using a rat model, and the regenerative capacity of a non-union bone defect using a rabbit model. Until now, a 3-D SWCNT/PLAGA composite was unavailable. The 3-D design of the SWCNT/PLAGA composite allows for greater porosity and provides a means of fixation in a large bone defect. Composites capable of promoting bone growth in a large bone defect will have a significant impact on bone 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.