Award Submission: Enhanced Cardiomyocyte Function On Poly-Lactic-Co-Glycolic Acid: Carbon Nanofiber Composites Under Electrical Stimulation

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Introduction:

Scarred cardiac muscle results in heart failure for millions of heart attack survivors worldwide. In 2009, an estimated 785, 000 Americans had a new coronary attack and about 470, 000 will have a recurrent heart attack leading to a coronary event. In recent years, various techniques have been developed to promote cardiomyocyte cell growth around dead tissue after a myocardial infarction. However, one area that has been largely omitted to date is the exploration of nanotechnology (or materials, so called nano-materials, with dimensions less than 1 micron) in cardiovascular applications.

In recent research, it was demonstrated that the use of nano-materials can promote the growth of cardiomyocytes when compared to the use of conventional or micro-structured materials. One such nano-material consisted of poly-lactic-co-glycolic acid (PLGA) with carbon nanofibers (CNFs) where it was shown that cardiomyocytes proliferated faster on the composites compared to a pure PLGA film after 5 days.

The object of this study was to better understand the cytocompatibility properties of this composite material for myocardial applications by using electrical stimulation which mimics that of the heart. For this reason, an *in vitro* continuous electrical stimulation model was used to determine cardiomyocyte functions on PLGA: CNF composite materials.

Methods:

A 4-Point conductivity method, scanning electron microscopy (SEM), Raman Spectroscopy, atomic force microscopy (AFM) and X-Ray Diffraction were used to characterize the materials after preparation. This consisted of using purified CNFs (99.9% by weight %, Catalytic Materials, MA) with a diameter of 100 nm and sonicated in 20 ml of chloroform at 20W for 30 minutes. Two pellets of PLGA (50:50 PLA:PGA wt.) (Polyscience Cat #23986) were diluted in a 50 ml flask with 30 ml of tetrahydrofuran and sonicated in a water bath below 30 ^{0}C for thirty minutes. After the PLGA and CNF solutions were prepared, various PLGA:CNF weight percent ratios were created (100:0, 75:25, 50:50, 25:75, 0:100) by adding the appropriate amount of CNF to PLGA in 20 ml disposable scintillation vials. When the appropriate ratios were reached, each composite material was sonicated at 10W for 20 minutes and vacuum dried for 48 hours.

To investigate cytocompatibility properties, human cardiomyocytes (Celprogen, Cat #36044-15, USA) were seeded onto PLGA:CNF composites in complete growth media supplemented with fetal bovine serum and antibiotics (Celprogen, Cat #M36044-15S, USA) at a density of 10 x 10^4 cells/cm^2 and were continuously stimulated (rectangular, 2 nm, 5 , 1 Hz) for 1, 3, and 5 days. MTT and Triponan I ELISA assays were completed to analyze cytocompatibility and cell viability on the composite. All experiments were performed at least in triplicate and results were compared to their non-electrically stimulated PLGA:CNF composite counterparts. When data were compared, ANOVA software and a student T-test were used. A p-value of < 0.05 was considered to be significant.

Results and Discussions:

Results of this study provided evidence that increasing the CNF weight ratio in PLGA increased conductivity. Scanning electron microscopy showed CNFs were uniformly dispersed within the PLGA matrix and, as expected, more CNFs were observed for the higher CNF ratio samples.

X-Ray diffraction spectra obtained from the as-synthesized PLGA:CNF composites confirmed its amorphous nature and complimentary evidence of the confirmation of the crystallinity was obtained using Raman spectroscopy.

Electrical stimulation increased cardiomyocyte density on all PLGA:CNF composite ratios as well as cardiac protein biomarker Troponin I (with 50:50 [PLGA:CNF (wt:wt) composite with the highest cell density and Troponin I levels) when compared to non-electrical stimulation data. Thus indicating that PLGA:CNF composites promote cardiomyocyte proliferation and differentiation in an electrically stimulated human heart environment as well as increased heart cell viability via increasing Troponin I protein synthesis.

Conclusions:

The present work demonstrates that a simple solution-mixing-drying based synthesis route can be adopted to develop PLGA:CNF hybrid biocomposites over a broad composition range possessing a uniform distribution of CNF without any clustering and using electrical stimulation to mimic heart conditions to promote cardiomyocyte growth. Electrical stimulation increased cardiomyocyte density on all PLGA:CNF composite ratios as well as cardiac protein biomarker Troponin I (with 50:50 [PLGA:CNF (wt:wt)] composite with the highest cell density and Troponin I levels) when compared to non-electrical stimulation data. This shows that PLGA:CNF composites can promote cardiomyocyte proliferation and differentiation in an *in vitro* heart model. Future work will consist of atomic force microscopy to better understand surface-cell interactions as well as surface conductivity analysis.

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